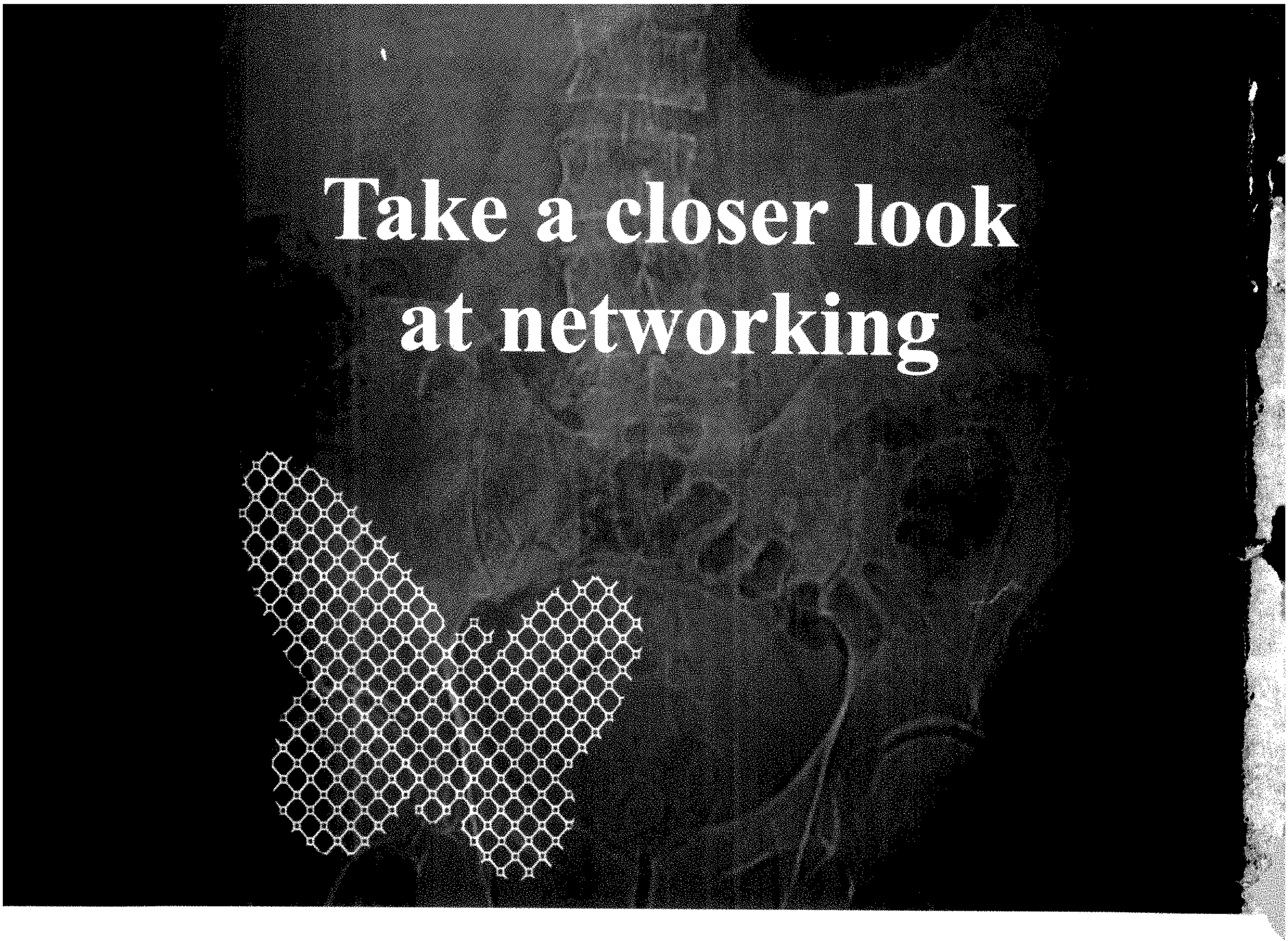

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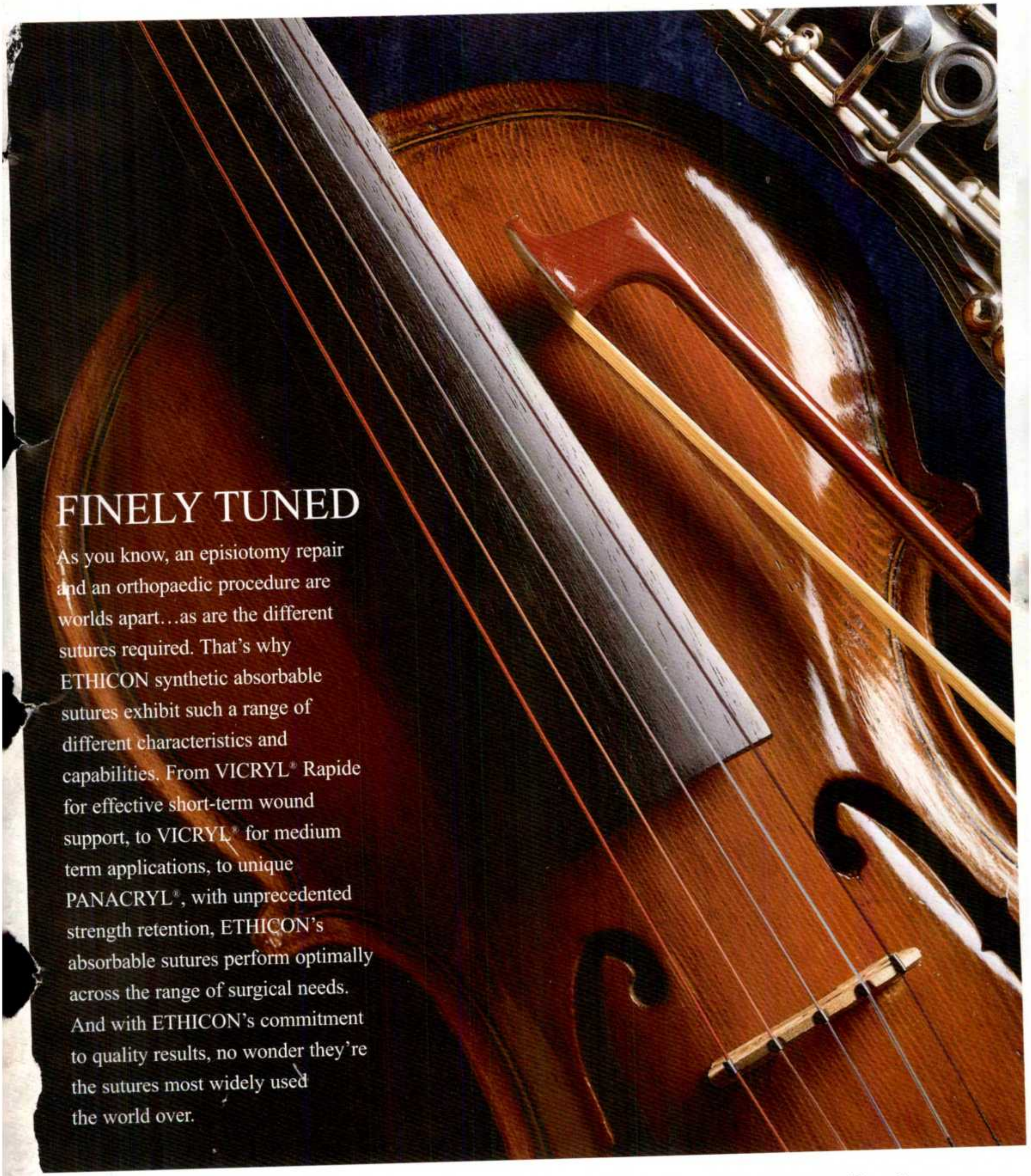

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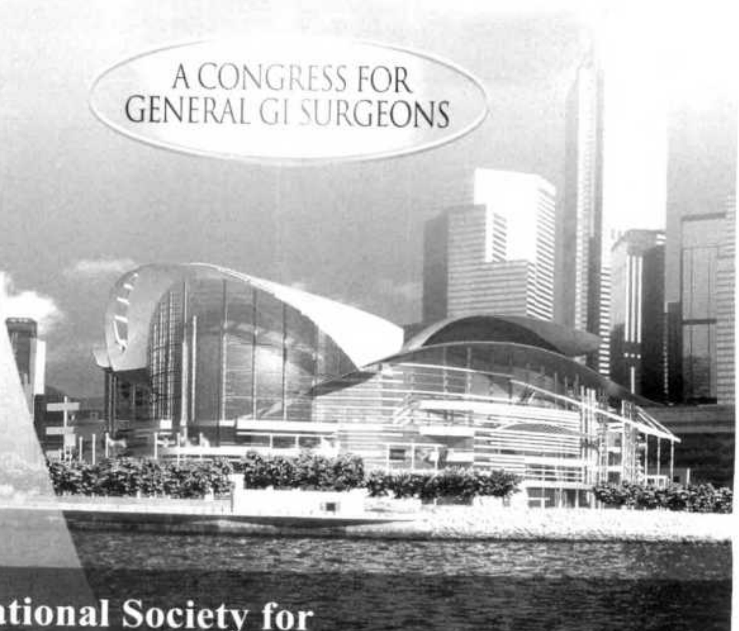

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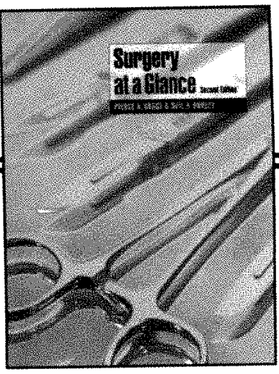

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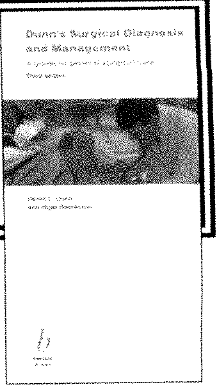

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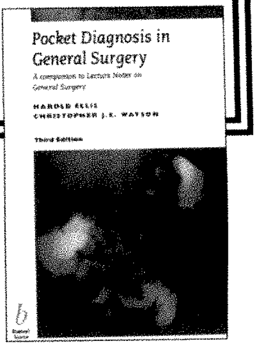
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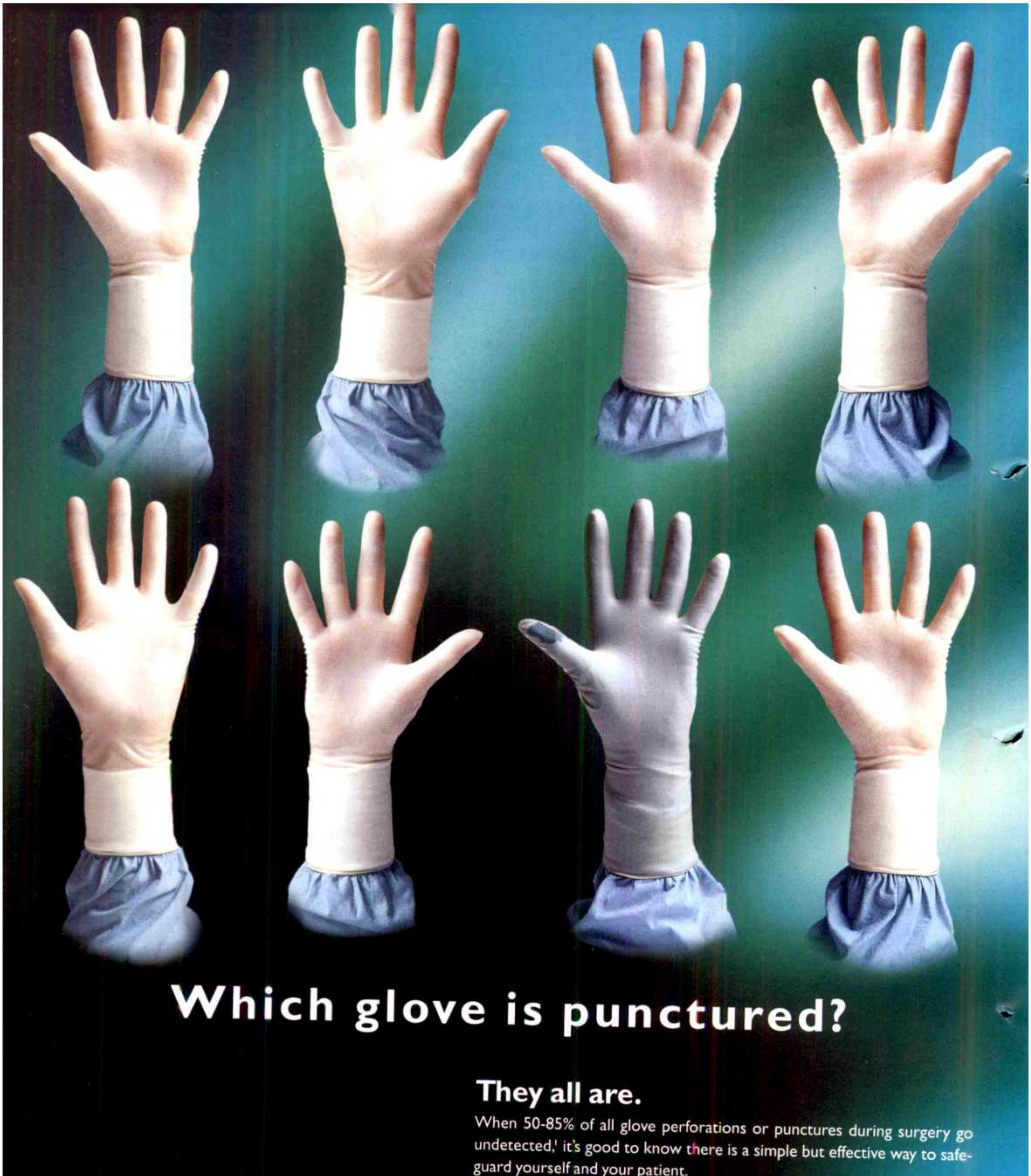

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Leading article

Management of intermittent claudication

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Intermittent claudication is the earliest clinical manifestation of peripheral arterial disease and is surprisingly common. The Edinburgh Artery Study found that 4 per cent of those aged over 55 years experienced symptoms, and asymptomatic disease was evident in a further 25 per cent[1]. Relatively few (2–8 per cent) claudicants progress to limb loss and 75 per cent remain symptomatically stable or improve. Of greater concern is the high associated cardiovascular risk; 50 per cent of claudicants die within 10 years, a relative risk of 3·8 compared with the normal population. Peripheral arterial disease is an independent risk factor for cardiovascular death and a claudicant is more likely to die from myocardial infarction than a similar patient with angina.

There is no consensus about the treatment of claudication and intervention rates vary greatly. The recent trend has been to a more conservative approach, but this has not been followed enthusiastically and in practice few patients receive the necessary advice and treatment. Claudication is a major disability and the impact on quality of life is similar to that of class 3 and 4 heart failure. A comparison of the resource available and enthusiasm with which patients with ischaemic heart disease are treated (many of whom are at less risk and less handicapped) with those for claudication is stark[2].

Non-interventional treatment

Risk factors

Evidence of the benefit of correction of associated risk factors is strong[3]. Smoking is the strongest risk factor for the development of claudication, smokers having three times the mortality rate of non-smokers. Smoking cessation lowers the mortality rate and is probably the most important action a claudicant can take. While there is no strong evidence that this will improve symptoms, the risk of developing critical limb ischaemia is reduced. Simple advice is not very effective, but support combined with adjunctive treatment, such as nicotine patches, can achieve a considerable reduction in the number of smokers.

In patients with established atheromatous vascular disease, aspirin reduces cardiovascular events significantly (27 per cent relative risk reduction)[4]. A large randomized clinical trial comparing aspirin with clopidogrel (an antiplatelet agent) showed a significant reduction in expected cardiovascular events, with clopidogrel achieving a slight advantage (relative risk reduction of 9 per cent). The subgroup with peripheral arterial disease appeared to gain the greatest benefit and there now seems little doubt that all claudicants should be on a suitable antiplatelet agent[5].

Lowering plasma lipid levels with drugs such as statins benefits patients at risk of cardiovascular events. Although the data largely apply to ischaemic heart disease, the benefit is greatest for patients with the highest cardiovascular risk rather than those with the highest baseline cholesterol level. Claudicants form one of the highest-risk groups and so intervention is justified; evidence from small studies supports this view. The current recommendation of treating only total cholesterol over 5·0 mmol/l has been challenged by evidence that statins may have a direct beneficial effect on atherosclerosis. Results from larger studies, currently under way, are needed to evaluate this in more detail, particularly the cost-effectiveness of more widespread use of these drugs[6].

Exercise

Exercise programmes address not only the presenting symptom of limited walking but may also reduce cardiovascular risk. No large single study has been undertaken, but a meta-analysis concluded that exercise can improve walking ability by approximately 150 per cent[7]. There is no clear evidence about the optimum form of exercise, but maximum benefit seems to be associated with supervised programmes of walking, at least three times per week over 6 months. While there is concern about the durability of benefit and compliance of patients outside a study environment, exercise programmes are economical and it is disappointing that only a quarter of consultant vascular surgeons in the UK have access to them.

Pharmacological treatment

Clinical trials have suggested improved walking ability from a number of drugs such as pentoxifylline and, more recently, the phosphodiesterase III inhibitor cilostazol. The clinical benefit is generally small and it is not clear if these agents are cost-effective. The favourable results of using ramipril in patients with peripheral arterial disease in the Heart

Outcomes Prevention Evaluation study may suggest other pharmacological ways of reducing the cardiovascular mortality rate.

Interventional treatment

Angioplasty

Percutaneous transluminal angioplasty (PTA) has some appeal as it is minimally invasive. This has reduced the threshold for intervention and a large number of angioplasties are undertaken for moderately severe claudication. Results vary considerably, but 90–100 per cent primary success rates with 60–80 per cent 1-year patencies have been reported for short superficial femoral artery occlusions, and successful angioplasty improves the quality of life of claudicants. The effect on symptoms is often not reported and there are few controlled studies comparing PTA with other treatments, especially exercise. Only two small randomized studies comparing PTA with exercise have been reported and both failed to show any long-term benefit from angioplasty. PTA, although less invasive than surgery, has a complication rate of 3 per cent, including a small risk of limb loss. If claudicants are investigated with either duplex ultrasonography or angiography, as few as 10 per cent are found suitable for conventional angioplasty; a considerable use of resource for little clinical benefit.

Surgery

Initial enthusiasm for bypass surgery has been tempered by concern over associated mortality and morbidity, including limb loss if the graft fails, and cost. Despite this, a considerable number of bypass procedures are still undertaken for claudication. For example, between 1995 and 2000 over 3000 such bypasses were recorded in the Swedish Vascular Registry (Swedvasc). In the largest randomized study of intervention in claudicants, surgery improved walking distance and quality of life significantly more than supervised exercise or controls[8,9]. Furthermore, no excess mortality or limb loss was found in the surgery group and ankle arterial pressure was significantly increased following intervention. The treatment effects were small, however, and compliance with the exercise programme was poor, weakening the conclusions; no attempt was made to assess the cost-effectiveness of the treatments. Disappointingly, combining exercise with surgery did not seem to confer any advantage. To date, there is no evidence of a clear role for bypass surgery or, perhaps more importantly, of a means of identifying patient groups that may benefit[10].

Future management of intermittent claudication

For such a common and significant condition it is disappointing that no consensus has been reached about what treatment a claudicant should expect. Such consensus has been successfully achieved in other areas, such as coronary heart disease, in the form of UK National Service Frameworks (NSFs) which are likely to benefit patients greatly. The NSF is soon to be released for diabetes, a condition far less prevalent than peripheral arterial disease. With such a wide range in attitude to the management of claudication it is not really surprising that the condition fails to achieve the awareness and support that patients deserve.

The evidence for many aspects of risk factor management seems compelling and yet most patients are still not receiving appropriate help. The real challenge seems to be in developing cost-effective ways of delivering these programmes and achieving good patient compliance. Conventional vascular clinics and general practitioner surgeries are not succeeding; community-based diagnostic and support clinics may provide more successful and appropriate treatment. This approach would also reduce the workload of vascular surgeons and general practitioners, allowing them to concentrate on those patients who may benefit from more aggressive treatment.

A broad role for interventional treatment is difficult to justify on current evidence, especially when cost is taken into account. While those studies that have been undertaken seem to suggest that both angioplasty and surgery may benefit small subgroups of patients, unless high-quality evaluation is done in more detail these relatively expensive and invasive treatments may lose support.

In the UK vascular surgeons tend to see the majority of claudicants referred from primary care for diagnostic and therapeutic consideration. In many other European countries these patients would initially see a medical angiologist. Only a small minority of patients will require intervention, so are vascular surgeons the appropriate specialists to manage claudication? Aspects of treatment encompass a range of medical specialties, but none has a major primary interest in peripheral arterial disease (there are very few angiologists in the UK). At present, I believe vascular surgeons are the group best placed to help direct and improve the treatment of claudication, but they need to be open to new ways of achieving this, particularly in conjunction with primary care. Claudication should no longer be left as the poor relation of coronary heart disease.

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Leading article

Oncoplastic surgery of the breast

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Cancer of the breast is the commonest malignancy in women in the Western world and a major cause of premature death; one in 11 women in the UK will be affected and the incidence is rising by about 2 per cent per year[1]. Paradoxically, mortality rates are decreasing and survival is improving, putatively due to a combination of earlier diagnosis, improved adjuvant endocrine therapy, chemotherapy and radiotherapy, and better surgical techniques. Randomized controlled trials of breast cancer management have played an important role.

Many women express a fear of breast cancer surgery that is based on the previous experience of an affected close relative; radical mastectomy with its attendant morbidity, and aesthetic and functional disfigurement, held sway for the best part of a century. Two decades ago, the hypothesis of breast cancer as a systemic disease from the outset stymied surgical focus and operative innovation, and led to a minimalist surgical approach in some centres. Recent data on the significance of recurrent disease indicate that, for an unknown but significant proportion of women, the systemic disease hypothesis is not appropriate, and so the role of surgery becomes increasingly important, especially with earlier cancer diagnosis. It is sometimes forgotten that the benefits of adjuvant treatment are in addition to those already conferred by operation.

Nevertheless, to obtain cancer ablation and control, surgery in the broadest sense is still 'radical'. Wide excision of a tumour may involve the removal of up to 20 per cent of breast volume, and be followed by radiotherapy; poor aesthetic outcomes after breast cancer surgery have not always been limited to those who have undergone mastectomy. Fortunately a woman need no longer put up with a gross deformity that may be a constant daily reminder of her diagnosis, limiting her choice of clothing and adversely affecting her quality of life.

Better survival, quality of life issues and increased expectations on behalf of both patient and surgeon have led to a surge of breast reconstruction techniques. Traditionally the general surgeon managed breast cancer, and the plastic surgeon undertook delayed breast reconstruction; either or both may have had little insight into the aims and difficulties of management faced by the other. Patient care has been enhanced by the development of the multidisciplinary team and the emergence of the specialist breast surgeon from both the traditional surgical specialties. The 'one size fits all' philosophy for both the oncological and the reconstructive elements of breast surgery has been abandoned. The result is seamless specialist breast cancer surgery in which ablation of the cancer is merged with reconstruction as an integrated whole. While individual surgeons with particular training and experience may provide the complete oncoplastic remit, care is more often shared between two surgeons working together within a breast cancer unit. In the UK, the Department of Health now provides significant funding for innovative cross-specialty training in oncoplastic breast surgery.

Four features are integral to oncoplastic breast surgery: appropriate surgery to extirpate the cancer, partial reconstruction to correct wide excision defects, immediate reconstruction using the full range of available techniques, and correction of asymmetry relative to the contralateral breast. All of these require planning with respect to the placement and closure of wounds, and careful assessment of the oncological need and aesthetic aim.

After breast-conserving cancer surgery and subsequent radiotherapy, up to 30 per cent of women have a residual deformity that may require surgical correction[2]. A few will have asymmetry without deformity that may be acceptable and require no revision, but another small group will have asymmetry with deformity of sufficient severity to warrant completion mastectomy and reconstruction with a myocutaneous flap. Evaluation of such deformity is of necessity a *post hoc* activity. Optimal treatment should be preventive – by performing immediate remodelling of the breast at the same time as cancer removal, before radiotherapy. Plastic surgical techniques may be integrated with the original operation, thereby reducing the need for delayed secondary procedures.

Moderate infill techniques involve the transfer of adjacent breast parenchyma and skin, such as the lunate flap[3]. More extensive replacement requires total breast remodelling, as in the therapeutic mammoplasty[4] or in the round block technique of central segmentectomy including the nipple–areola complex[5]. Therapeutic mammoplasty often requires contralateral breast reduction; this can be a cause of dissatisfaction if breast volume is limited. The latissimus dorsi miniflap to infill resections of more than 25 per cent of breast volume allows breast conservation that otherwise would be impossible from an oncological standpoint[6,7]. Such approaches extend the spectrum of breast-conserving cancer management.

Up to 40 per cent of patients diagnosed with breast cancer still undergo mastectomy because of multifocal pathology, adverse tumour : breast size ratio or site, or through choice. Immediate breast reconstruction, achieved in a single process combined with mastectomy, has increased over the past few years owing to recognition of its oncological safety for selected patients, the development of reliable, technically and aesthetically sophisticated reconstruction operations[8], and its cost-effectiveness. Better aesthetic and functional results of the latissimus dorsi flap result from latissimus dorsi tendon division at the humeral groove, and increasing acceptance of the use of an implant deep to the flap. The transverse rectus abdominis flap, both pedicled and free, provides good immediate breast replacement for selected patients, but the abdominal scarring and possible sensory and functional sequelae need careful consideration and discussion. For both main flaps, the evolution of skin-sparing mastectomy techniques has significantly improved appearance and sensation[9], and the easily achieved nipple–areola complex reconstruction using a combination of small local flaps and tattoo completes the result. The appearance of a reconstruction performed as a delayed technique deteriorates over time[10]; there are no equivalent data yet available on long-term outcome after skin-sparing mastectomy and immediate reconstruction.

Oncoplastic surgery of the breast is an innovative, progressive and sophisticated subspecialty with new cross-specialty training opportunities. However, it lacks published randomized clinical trials comparing surgical techniques and objective measures of functional and aesthetic outcome. Increasing attention to breast conservation should, in time, allow this defect to be addressed.

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Review

Surgical treatment of incisional hernia

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Background: Incisional hernia is a common complication of abdominal surgery and an important source of morbidity. It may be repaired using open suture, open mesh or laparoscopic mesh techniques. This review examines the results of these methods of repair.

Methods: A Medline literature search was performed to identify articles relating to 'incisional hernia', 'ventral hernia' and 'wound failure'. Relevant papers from the reference lists of these articles were also sought.

Results: The recurrence rate after open suture repair may be as high as 31–49 per cent; for open mesh repair it is between 0 and 10 per cent. Comparative studies show that recurrence is significantly more frequent after open suture repair than after open mesh repair; complication rates are similar for both procedures. Recurrence rates after laparoscopic mesh repair vary from 0 to 9 per cent. Comparative studies show that laparoscopic mesh repair is at least as safe and effective as open mesh repair.

Conclusion: Open suture repair for incisional hernia carries an unacceptably high recurrence rate. The results of open mesh and laparoscopic mesh techniques are encouraging. There is scope for a large multicentre randomized clinical trial to compare laparoscopic and open mesh repair.

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Introduction

An incisional hernia has been defined as a bulge visible and palpable when the patient is standing and often requiring support or repair[1]. It is a common complication of abdominal surgery, reported in up to 11 per cent of patients generally[2,3] and in up to 23 per cent of those who develop postoperative wound infection[4]. It is an important source of morbidity. A considerable proportion of patients present with incarceration and strangulation, requiring emergency abdominal surgery[5]. Others may need to alter their lifestyle, or change or give up gainful employment[6], which results in an economic burden that has not been fully evaluated.

Treatment involves further major surgery and the results may be poor, with recurrence rates of up to 49 per cent reported[7]. These high recurrence rates prompted recommendations of 'a cautious attitude to surgical treatment of incisional hernia' in the mid-1980s[8]. Since then, in spite of the frequency of the condition and its potential morbidity, no consensus on the best treatment has been forthcoming[9]. A wide spectrum of surgical techniques has been developed and recommended, ranging from sutured techniques to the use of various types of prosthetic mesh. Laparoscopic repair was a novel approach introduced in the 1990s. This review article examines the results of open sutured, open mesh and laparoscopic mesh repair of incisional hernia.

Open suture repair

Before the introduction of knitted polypropylene mesh in the early 1960s, most incisional hernias were repaired by direct suture techniques. These included simple fascial closure (*Fig. 1*), modified Mayo technique with overlap of fascial edges, use of internal retention sutures[10], 'keel' procedure using relaxing incisions in the lateral aspect of the anterior rectus sheath[11], the Nuttall procedure[11] involving transposition of rectus abdominis and its enveloping fascia, use of layered steel wire[12] and others. Such proliferation of techniques is a typical result of overall poor outcome. Recurrence rates of between 7 and 44 per cent were reported until 1980 (*Table 1*), the only exception being one study from the Shouldice Clinic, in which layered steel wire was used, with a reported recurrence rate of 2 per cent[12]. Such an inflexible material was, however, difficult to handle and its use was abandoned.

In spite of these poor results suture techniques have continued to be used (*Table 2*). The larger studies have described very high rates of recurrence of between 31 and 49

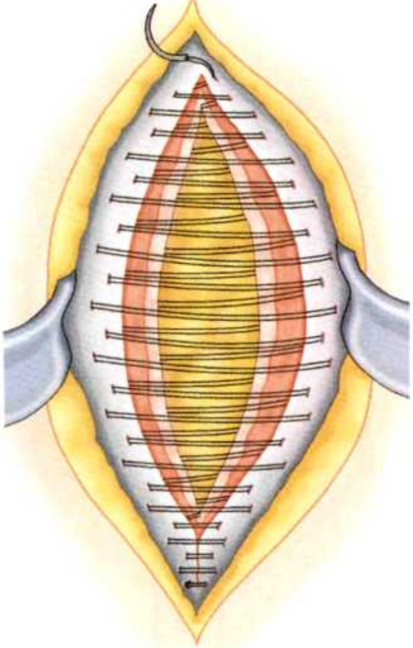

Fig. 1 Simple fascial closure

per cent[7,8,20,22]. George and Ellis[20] argued that the underlying problem was that in all suture repair techniques the tissues are under tension and this increases the risk of ischaemia, suture cut-out and repair failure.

However, Naraynsingh and Ariyanayagam[21] reported only one recurrence (1 per cent) among 85 patients treated with a 'keel' technique (*Fig. 2*). The hernial sac was dissected free and simply reduced without dissection of

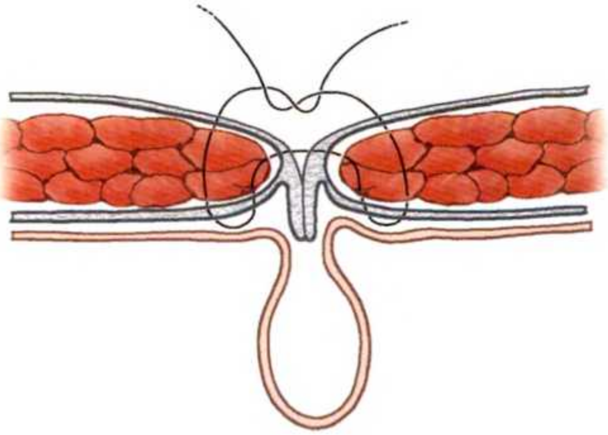

Fig. 2 Keel repair

Table 1 Recurrence rates after incisional hernia repair using open suture repair techniques (studies published up to 1980)

Reference	Year	Technique	No. of patients	Recurrence rate (%)
Obney[13]	1957	Layered steel wire	192	12
Young[14]	1961	Rectus relieving incision	15	7
Akman[12]	1962	Layered steel wire	500	2
Horton and Smith[15]	1969	Various techniques	36	44
Fischer and Turner[16]	1974	Various techniques	151	17
Maguire and Young[17]	1976	Rectus relieving incision	32	19
Jenkins[18]	1980	Mass nylon	50	8

Table 2 Recurrence rates after incisional hernia repair using open suture repair techniques (studies published since 1980).

Reference	Year	Technique	No. of patients	Recurrence (%)	Follow-up (months)
Hope *et al.*[19]	1985	Da Silva method	27	0	30
Langer and Christiansen[8]	1985	Primary closure	154	31	48–120
George and Ellis[20]	1986	Keel or mass nylon	81	46	14
van der Linden and van Vroonhoven[7]	1988	Primary closure, two-layer fascial approximation, Mayo overlap repair	151	49	39
Naraynsingh and Ariyanayagam[21]	1993	Keel technique	85*	1	71
Gecim *et al.*[22]	1996	Primary closure	109†	45	7–92
Kuzbari *et al.*[23]	1998	Sliding door technique	10	0	26–66
Shukla *et al.*[24]	1998	Cardiff repair	50	0	52

*Only 23 had an incisional hernia; the remainder had paraumbilical, umbilical and epigastric hernias. †Nine underwent mesh repair sac contents. Both rectus abdominis muscles were exposed for at least 3 cm from their edge. An inverting suture technique was then used to bring the muscle bellies together in the midline to close the hernial defect and invert the hernial sac in a 'keel' fashion. Loop nylon was used. However, of the 85 patients only 23 had an incisional hernia; 44 had a paraumbilical, 11 had an umbilical and seven had an epigastric hernia. Assuming that the single recurrence occurred in one of the 23 patients undergoing incisional hernia repair, the recurrence rate would still be a very acceptable 4 per cent. The authors suggested that their good results stemmed from the fact that their technique approximated muscle rather than fibroaponeurotic linea alba or anterior rectus sheath, both of which they claimed become stretched beyond their elastic limits, predisposing to herniation. Muscle, on the other hand, maintains an isometric state with varying load.

Shukla and colleagues[24] reported no recurrence in 50 patients followed up for a mean of 52 months. The sac was excised and the peritoneum closed before a 'Cardiff far and near' primary abdominal wall closure was performed. However, the authors only recommended this technique for 'small and medium sized defects'.

Two smaller studies[19,23] reported no recurrences. The first[19] included 27 patients who underwent a da Silva repair; using flaps of rectus sheath and fibroperitoneal tissue from the sac itself, three layers were produced in front of the pre-existing abdominal wall defect. Loop nylon was used for the repair. The good results obtained contradicted the suggestion of Naraynsingh and Ariyanayagam[21] that approximating aponeurotic tissue for repair would lead to high recurrence rates. There were, however, three deaths giving a mortality rate of 11 per cent and a complication rate of 78 per cent. The other study[23], which included only ten patients, used a 'sliding door technique', involving relaxing incisions in the aponeuroses of external oblique and overlapping rectus abdominis muscles. The good results of both of these studies have not, however, been reproduced in any large trial.

The commonest complications after open suture repair are wound related (*Table 3*) and include wound infection, haematoma, stitch sinus and flap necrosis. These occurred in 10–44 per cent of cases. Kuzbari and colleagues[23] reported one seroma in ten patients undergoing suture repair. Some of the 21 patients (26 per cent) who developed wound complications in the study by George and Ellis[20] had a seroma but the exact number is not specified in the report. Hope and co-workers[19] reported three deaths (11 per cent), one from coronary thrombosis, one from sepsis related to simultaneous open cystolithotomy and one from carcinomatosis. No death was reported in the other studies. Only Hope *et al.*[19] reported any respiratory complications; 12 of 27 patients developed respiratory problems of whom three required ventilatory support.

Open mesh repair

Usher[25] introduced knitted monofilament polypropylene (Marlex; C. R. Bard, Billerica, Massachusetts, USA) mesh into clinical practice in 1963. Other types of mesh have been used since then, although the two most commonly used remain polypropylene and expanded polytetrafluoroethylene. Various studies of mesh repair of incisional hernia have been reported in the English language literature (*Table 4*). The recurrence rates vary between 0 and 10 per cent with a follow-up period of at least 12 months, with one exception[38] when patients were followed up for between 6 and 33 months. This is markedly less than recurrence rates reported for suture repair.

The techniques used vary considerably (*Table 5*). In most of the studies the hernial sac was dissected and opened to allow division of adhesions before the contents of the sac could be reduced into the abdomen. Opening of the sac is an essential part of the procedure when the mesh is placed

Table 3 Complications of open suture repair

Reference	Year	No. of patients	No. of seromas	Wound complications	Other complications
Hope *et al.*[19]	1985	27	0 (0)	12 (44)	3 (11) deaths 12 (44) respiratory
Langer and Christiansen[8]	1985	154	0 (0)	64 (42)	11 (7) relaparotomy
George and Ellis[20]	1986	81	–	21 (26)	–
van der Linden and van Vroonhoven[7]	1988	151	0 (0)	24 (16)	–
Naraynsingh and Ariyanayagam[21]	1993	85	–	–	–
Gecim *et al.*[22]	1996	109*	0 (0)	26 (24)	–
Kuzbari *et al.*[23]	1998	10	1 (10)	1 (10)	–
Shukla *et al.*[24]	1998	50	0 (0)	6 (12)	–

Values in parentheses are percentages. *Eight underwent mesh repair intraperitoneally (*Fig. 3*), but some surgeons who insert the mesh extraperitoneally (*Fig. 3*) also open the sac, not only to divide adhesions but also to enable digital exploration of the interior of the abdominal wall to ensure there are no other defects[26].

Those preferring not to open the sac[28,33,40] argue that this decreases the risk of bowel injury, fistula, development of postoperative ileus and bleeding. Bauer and colleagues[35] reported three fistulas, all of which followed opening the peritoneum and dissecting off small bowel resulting in enteric injury. In all three cases this was necessary to remove a previously implanted prosthesis. McLanahan and colleagues[30] and Balen and co-workers[31] reported one fistula each in their studies of 106 and 45 patients respectively.

Table 4 Recurrence rates after incisional hernia repair using open mesh techniques

Reference	Year	Mesh used	No. of patients	Recurrence (%)	Follow-up (months)
McCarthy and Twiest[26]	1981	Polypropylene	25	8	27
Matapurkar *et al.*[27]	1991	Polypropylene	60	0	36–84
Temudom *et al.*[28]	1996	Polypropylene and PTFE	50	4	24
Gillion *et al.*[29]	1997	PTFE	158	4	37
McLanahan *et al.*[30]	1997	Polypropylene	106	4	24
Balen *et al.*[31]	1998	PTFE	45	2	39
Turkcapar *et al.*[32]	1998	Polypropylene	45	2	36
Whiteley *et al.*[33]	1998	Polypropylene	10	0	17
Arnaud *et al.*[34]	1999	Dacron	250	3	97
Bauer *et al.*[35]	1999	PTFE	98	10	72
Utrera Gonzalez *et al.*[36]	1999	PTFE	84	2	12–36
Chrysos *et al.*[37]	2000	PTFE	52	8	–
Ladurner *et al.*[38]	2001	Polypropylene	57	2	6–33
Martin-Duce *et al.*[39]	2001	Polypropylene	152	1	72

PTFE, polytetrafluoroethylene

Table 5 Studies of open mesh repair techniques

Reference	Year	No. of patients	Recurrence (%)	Hernial sac	Type of mesh	Site of mesh and overlap	Suture type	Drains
McCarthy and Twiest[26]	1981	25	8	Opened, sac excised	Polypropylene	Intraperitoneal 3 cm	Polypropylene	Suction
Matapurkar *et al.*[27]	1991	60	0	Opened, not excised	Polypropylene	Extraperitoneal 1–2 cm	Polypropylene	Suction + corrugated
Temudom *et al.*[28]	1996	50	4	Reduced, not opened	Polypropylene	Extraperitoneal 4–6 cm	Polydioxanone/ polypropylene	Suction
McLanahan *et al.*[30]	1997	106	4	Opened, excised	Polypropylene	Extraperitoneal 4–6 cm	Absorbable	Jackson-Pratt
Whiteley *et al.*[33]	1998	10	0	Reduced, not opened	Polypropylene	Extraperitoneal 0 cm to edge	Nylon/ polypropylene	Suction
Balen *et al.*[31]	1998	45	2	Opened if necessary	PTFE	Extraperitoneal 2 cm	Polygluconate	Suction
Bauer *et al.*[35]	1999	98	10	Opened, not excised	PTFE	Extraperitoneal Three techniques	Unknown	No drains
Utrera Gonzalez *et al.*[36]	1999	84	2	Opened, not excised	PTFE	Intraperitoneal 5–7 cm	Unknown	Suction
Arnaud *et al.*[34]	1999	250	3	Opened, excised	Dacron™*	Intraperitoneal 10 cm	Non-absorbable	4†
Luijenkijk *et al.*[40]	2000	84	23	Reduced	Polypropylene	Extraperitoneal 2–4 cm	Polypropylene	Optional
Ladurner *et al.*[38]	2001	57	2	Opened, excised	Polypropylene	Extraperitoneal 'far beyond edges'	Unknown	Unknown
Martin-Duce *et al.*[39]	2001	152	1	Opened, not excised	Polypropylene	Extraperitoneal	Polyglactin	Suction

PTFE, polytetrafluoroethylene. *Dupont, Wilmington, Delaware, USA. †Type not stated

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Preserving the hernia sac also provides a layer of viable autogenous tissue to serve as a barrier between the prosthesis and the intraperitoneal contents, possibly decreasing the risk of adhesions, intestinal obstruction and fistula[41]. Indeed some of those who open the sac and dissect adhesions subsequently resuture the peritoneum to provide such a barrier. When this is not possible, omentum may be interposed between the mesh and abdominal contents, or a polyglactin mesh may be fixed to the undersurface of the permanent mesh[40].

On the other hand, intraperitoneal placement of mesh[26,34,36] using the open technique and, more recently, laparoscopically, has yielded good results with no apparent increase in complications. Initial concern about complications associated with intraperitoneal polypropylene mesh placement[42–45] now seem to be subsiding, although the debate persists. Indeed Vrijland *et al.*[46] concluded that enterocutaneous fistula formation appears to be very rare after incisional hernia repair with polypropylene mesh, regardless of intraperitoneal placement, omental coverage or closing of the peritoneum.

Satisfactory results have been obtained with onlay[35], extraperitoneal[33,35], subfascial[27–29,35,38–40] and intraperitoneal[26,29,36] techniques (*Fig. 3*). Most surgeons have used non-absorbable sutures (mainly polypropylene) to anchor the mesh. However Martin-Duce and colleagues[39] claimed that use of non-absorbable sutures to fix the prosthesis resulted in high tension and explained the high incidence of abdominal pain reported[28,38,39]. They felt that using absorbable sutures (polyglactin) reduced this problem.

There appears to be general agreement on the need to achieve overlap of the mesh with the abdominal tissue by at least 3 cm[26,28,30,34–36,38–40]. This allows a large surface area laterally for ingrowth of connective tissue, leading to permanent fixation of the prosthesis within the abdominal

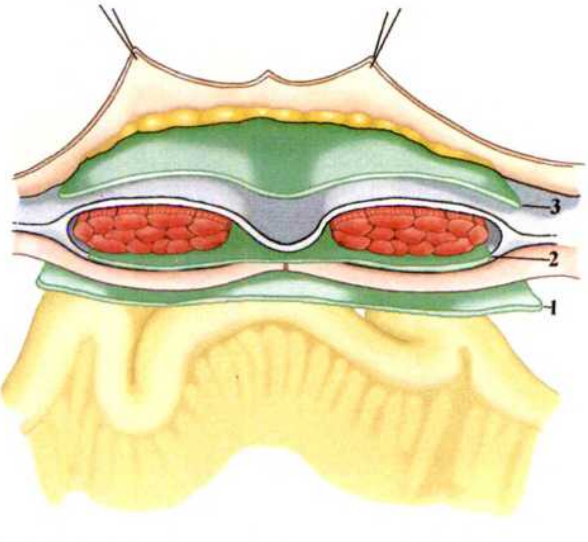

Fig. 3 Options for position of mesh. 1, Intraperitoneal; 2, extraperitoneal/subfascial; 3, onlay

Table 6 Complications with open mesh repair

Reference	Year	No. of patients	No. of seromas	Wound complications	Ileus	Pain	Other
McCarthy and Twiest[26]	1981	25	–	1 (4)	–	–	–
Matapurkar *et al.*[27]	1991	60	–	10 (17)	2 (3)	1 (2)	–
Temudom *et al.*[28]	1996	50	3 (6)	8 (16)	1 (2)	7 (14)	–
Gillion *et al.*[29]	1997	158	–	–	–	–	5 (3) sepsis
McLanahan *et al.*[30]	1997	106	1 (1)	19 (18)	–	5 (5)	1 (1) fistula
Turkcapar *et al.*[32]	1998	45	2 (4)	4 (9)	–	–	–
Balen *et al.*[31]	1998	45	2 (4)	2 (4)	–	–	1 (2) fistula 1 (2) wound sinus
Whiteley *et al.*[33]	1998	10	–	0 (0)	–	–	–
Bauer *et al.*[35]	1999	98	5 (5)	9 (9)	–	–	3 (3) fistula
Utrera Gonzalez *et al.*[36]	1999	84	5 (6)	6 (7)	–	–	19 (19) explantation 2 (2) respiratory failure 1 (1) death from necrotizing fasciitis
Arnaud *et al.*[34]	1999	250	–	10 (4)	–	–	2 (1) deaths
Chrysos *et al.*[37]	2000	52	8 (15)	4 (8)	–	–	–
Ladurner *et al.*[38]	2001	57	–	6 (11)	–	12 (21)	1 (2) death from pulmonary embolism
Martin-Duce *et al.*[39]	2001	152	14 (9)	24 (16)	3 (2)	42 (28)	1 (1) deep vein thrombosis 3 (2) urinary infection 2 (1) respiratory infection

Values in parentheses are percentages

wall[36,40]. The wide placement also decreases the risk of recurrent hernia occurring at the lateral edge of the defect. The wide overlap also allows for the shrinkage of the mesh that has been shown to occur in experimental *in vivo* studies in dogs[47].

In most of the studies reported, suction drains have been used in an attempt to prevent seroma and haematoma formation, although one group[39] stressed that vacuum-sustained drainage was turned off as early as possible in order to reduce the risk of infection. Vrijland and colleagues[46], however, concluded that the present results suggest no relation between insertion of a subcutaneous drain and a decrease in seroma formation.

Seroma formation occurred in 1–15 per cent of cases (*Table 6*) Wound infection was another common complication (4–12 per cent), in some cases requiring removal of the mesh[29,37,39]. Bauer and colleagues[35] reported a 3 per cent incidence of fistula, and Martin-Duce *et al.*[39] reported persistent pain beyond 6 months in 28 per cent of patients.

Heavy contamination at the time of surgery significantly increases the risk of severe complications when mesh is used. One study[48] using polypropylene mesh described gross contamination in 29 of 31 patients, 14 of whom ultimately extruded the mesh. The authors recommended avoiding mesh in these circumstances.

Open suture repair *versus* open mesh repair

Five recent studies, one prospective[40] and four retrospective, have compared mesh repair and primary closure (*Table 7*). Liakakos and colleagues[49] found that the recurrence rate with mesh repair was only 8 per cent compared with 25 per cent after suture repair after 90 months of follow-up. Similarly, in a larger comparative study of 272 hernias, Schumpelick *et al.*[50] found a recurrence rate of 7 per cent for mesh repair and 33 per cent for suture repair after a mean follow-up period of 64 months. Koller and colleagues[51] retrospectively compared the results of sutured repair in 70 patients with mesh repair in 26 patients. The recurrence rate after 24 months was 63 per cent for the sutured group and 13 per cent for the mesh group. The recurrence rate for sutured repair is the highest rate reported in the literature. In the study by Clark[52], in which 13 patients had suture repair and eight had mesh repair, the recurrence rate was 38 per cent for the former group after 25 months and 25 per cent for the latter group after 13 months of follow-up.

The only randomized prospective trial comparing open suture repair and mesh repair was that reported by Luijendijk *et al.*[40]. This was a multicentre trial, which randomly assigned 200 patients to suture or mesh repair. A polypropylene mesh was used and this was sutured in a subfascial position. Of the 181 patients included in the study, 97 had suture repair and 84 mesh repair. The recurrence rate was 46 per cent in the suture group and 23 per cent in the mesh group ($P = 0.005$) after a mean duration of follow-up of 26 months. The complication rate did not appear to be significantly different between the two groups although this was not analysed statistically in the study.

Laparoscopic incisional hernia repair

Laparoscopic repair of incisional hernia was introduced in the 1990s, with the expectation that recurrence rates similar to those of open mesh repair could be obtained, with an improvement in recovery time, hospital stay and complication rate. Since 1996, 14 reports of laparoscopic repair have been published (*Table 8*), all placing mesh in an intraperitoneal position.

The techniques used in these studies were very similar. After achieving a pneumoperitoneum, a variable number of

Table 7 Studies comparing open suture and open mesh repair of incisional hernia

Reference	Year	Technique	No. of patients	Recurrence (%)	Follow-up (months)
Liakakos *et al.*[49]	1994	Suture repair	53	25	90
		Polypropylene mesh	49	8	90
Schumpelick *et al.*[50]	1996	Suture repair	190	33	64
		Polypropylene mesh	82	7	64
Koller *et al.*[51]	1997	Suture repair	70	63	24
		PTFE mesh	26	13	24
Clark[52]	2001	Suture repair	13	38	25
		Polypropylene mesh	8	25	13
Luijendijk *et al.*[40*]	2000	Suture repair	97	46	26
		Polypropylene mesh	84	23	26

*Randomized clinical trial. PTFE, polytetrafluoroethylene

Table 8 Studies of laparoscopic incisional hernia repair

Reference	Year	Type of mesh	No. of patients	Recurrence (%)	Follow-up (months)
Park *et al.*[53]	1996	PTFE/polypropylene	30	3	18
Costanza *et al.*[54]	1998	PTFE	16	5	18
Franklin *et al.*[55]	1998	Polypropylene	176	1	30
Toy *et al.*[56]	1998	PTFE	144	4	7
Koehler and Voeller[57]	1999	PTFE	32	9	20
Kyzer *et al.*[58]	1999	PTFE	53	2	17
Sanders *et al.*[59]	1999	PTFE	11	9	12
Carbajo *et al.*[60]	2000	PTFE	100	2	30
Chowbey *et al.*[61]	2000	Polypropylene	202	1	35
Heniford and Remshaw[62]	2000	PTFE	100	3	22
Heniford *et al.*[63]	2000	PTFE	407	3	23
Szymanski *et al.*[64]	2000	Polypropylene	44	5	7
LeBlanc *et al.*[65]	2001	PTFE	96	9	51
Moreno-Egea *et al.*[66]	2001	Parietex*	20	0	10

PTFE, expanded polytetrafluoroethylene. *Sofradim, Villefranche sur Soane, France

trocars were inserted. Most surgeons placed the trocars as far laterally from the hernial defect as possible[56,67–69]. The majority of surgeons preferred using a 30° or 45° laparoscope[56,63,68,69], while others used a 0° instrument[65]. Adhesions were divided and the contents of the hernial sac reduced. The edges of the defect or defects were exposed on all sides. In all cases the hernial sac was left *in situ*. Heniford and colleagues[63] felt that leaving the sac *in situ* did not give rise to problems other than to allow seroma formation during the first few weeks. Within approximately 6 weeks, however, all seromas in their study had resolved and only eight seromas were reported in the 407 patients (2 per cent). In other studies seromas occurred in between 1 and 36 per cent of patients[54–56,59,60,62,63,65].

Once the size of the hernial defect was defined, the proper size of mesh was determined. The prosthetic mesh was then inserted into the peritoneal cavity. There appears to be universal agreement that an overlap of at least 3 cm between the mesh and the fascial edge is necessary[55,56,59,62,63,65]. In the early studies, tacks, staples and even titanium screws were used to fix the mesh in place. However, staples alone have been found to provide inadequate fixation[56], and through and through sutures, inserted using a needle-point suture passer (*Fig. 4*), are now generally used[55,56,59,63,69]. The Gore suture passer[59,63] (W. L. Gore, Newark, Delaware, USA) and the Carter-Thomason Needle-Point suture passer[55] (Inlet Medical, Eden Prairie, Minnesota, USA) are the two most often used. The suture passer has a grasping jaw to carry a stitch via a small skin incision through the abdominal wall into the abdomen and through the mesh. The stitch is released and the passer is removed and reinserted via the same skin site to grasp the stitch about 1 cm away to create a mattress suture.

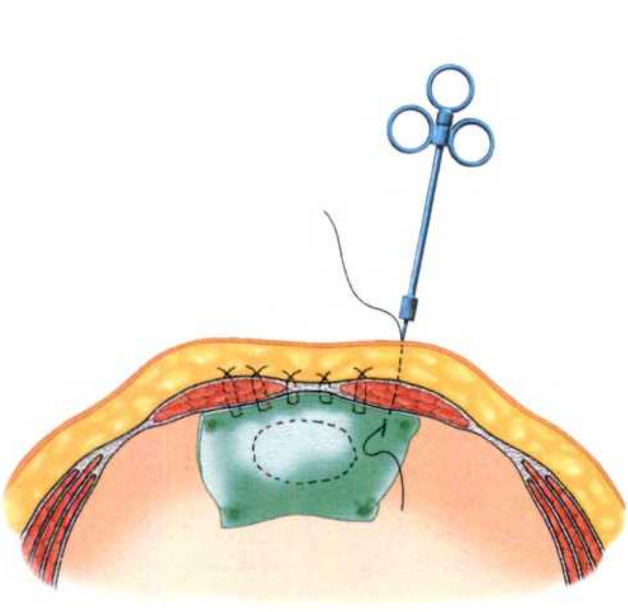

Fig. 4 Inserting sutures at laparoscopic mesh repair (broken line shows abdominal wall defect)

In one study[65] all nine recurrences in 96 patients treated by laparoscopic repair occurred in those in whom only tacks or staples, rather than sutures, had been used. The reported interval distance between sutures varies between 2 cm[59] and 5 cm[63], but tacks or staples are inserted between sutures and these should probably be no more than 1 cm apart. Most authors have not used drains, in contrast to the fashion with open mesh techniques where the use of drains is almost universal.

Table 9 Complications with laparoscopic incisional hernia repair

Reference	Year	No. of patients	Seroma	Wound complications	Ileus	Bowel injury	Prolonged pain	Other
Park *et al.*[53]	1996	30	–	1 (3)	3 (10)	–	–	–
Costanza *et al.*[54]	1998	16	2 (12)	–	–	–	–	–
Franklin *et al.*[55]	1998	176	2 (1)	3 (2)	–	–	3 (2)	–
Toy *et al.*[56]	1998	144	23 (17)	5 (4)	3 (2)	–	–	1 (1) bowel obstruction
Koehler and Voeller[57]	1999	32	–	2 (6)	–	2 (6)	–	1 (3) death
Kyzer *et al.*[58]	1999	53	–	1 (2)	–	2 (4)		2 (4) bowel obstruction
Sanders *et al.*[59]	1999	11	4 (36)	–	–	–	–	–
Carbajo *et al.*[60]	2000	100	10 (10)	5 (5)	–	–	–	–
Chowbey *et al.*[61]	2000	202	64 (32)	5 (2)	–	–	–	–
Heniford and Ramshaw[62]	2000	100	3 (3)	3 (3)	2 (2)	2 (2)	2 (2)	1 (1) urinary retention 1 (1) respiratory distress
Heniford *et al.*[63]	2000	407	8 (2)	8 (2)	9 (2)	5 (1)	8 (2)	4 (1) mesh infection 1 (0) intra-abdominal abscess 1 (0) trocar-site hernia
LeBlanc *et al.*[65]	2001	96	7 (7)	2 (2)	1 (1)	–	1 (1)	1 (1) trocar-site hernia
Moreno-Egea *et al.*[66]	2001	20	–	–	–	–	–	–

Values in parentheses are percentages

Table 10 Studies comparing laparoscopic mesh and open mesh incisional hernia repair

Reference	Year	Type of procedure	No. of patients	Recurrence (%)	Follow-up (months)
Holzman *et al.*[70]	1997	Open	16	12	19–20
		Laparoscopic	21	10	19–20
Park *et al.*[71]	1998	Open	49	34	53
		Laparoscopic	56	11	24
Carbajo *et al.*[72]*	1999	Open	30	7	27
		Laparoscopic	30	0	27
Ramshaw *et al.*[67]	1999	Open	174	21	21
		Laparoscopic	79	3	21
Chari *et al.*[68]	2000	Open	14	0	6–27
		Laparoscopic	14	0	6–27
Zanghi *et al.*[69]	2000	Open	15	0	40
		Laparoscopic	11	0	18

*Randomized clinical trial

The recurrence rates obtained with laparoscopic incisional hernia repair vary between 0 and 9 per cent. In four of the bigger studies[55,60,62,63], the recurrence rate was 1–3 per cent, with a follow-up period of at least 22·5 months, similar to rates described for open mesh repair with a shorter follow-up. In one of these studies[55], however, only 112 of the reported 176 patients had an incisional hernia, the rest being mainly umbilical. Common complications of laparoscopic repair include seroma, wound infection (including trocar-site infection), ileus, haematoma and pain (*Table 9*). In some cases infection led to removal of the mesh. More serious complications, such as small bowel injury, were less common (0–6 per cent).

Laparoscopic *versus* open incisional hernia repair

There are six studies in the literature comparing laparoscopic and open repair (*Table 10*), only one of which was a randomized clinical trial[72]. In the five retrospective studies[67–71] the rate of recurrence with laparoscopic mesh repair was the same as, or less than, that of open mesh repair, although in two of these studies the follow-up period was substantially longer for the open repair group. In another of these studies[70] the two groups were not comparable as the patients undergoing open repair were older and had larger fascial defects.

In the randomized clinical trial[72], 60 patients had either open or laparoscopic mesh repair. The recurrence rate in the open group was 7 per cent; there was no recurrence in the laparoscopic group over the same mean follow-up period of 27 months. Complications were fewer and the hospital stay was significantly shorter in the laparoscopic group, although the staff taking care of the patients were not blinded to the type of procedure. Operating time was also significantly shorter in the laparoscopic group. Ramshaw and colleagues[67] also found that operating time was shorter for the laparoscopic group (58 *versus* 82 min) but the other four comparative studies found the opposite.

Five of six studies[67,69–72] reported higher complication rates and longer hospital stay in the open group. Chari and colleagues[68] found no statistically significant difference between the two groups in terms of hospital stay or complication rate. The conclusion from all six studies was that laparoscopic incisional hernia is at least as effective and as safe as open mesh repair. Chari and colleagues[68] concluded that there was no demonstrable advantage of laparoscopic over open repair (although no demonstrable disadvantage either), while the other authors suggested that laparoscopic repair was better in terms of complications and duration of hospital stay.

Factors influencing recurrence

The most consistent risk factor for recurrence is wound infection[4,7,22,40,55]. Houck and colleagues[73] showed that open incisional hernia repair is associated with a significantly higher infection rate than other clean general surgical procedures, and so it is not surprising that the use of prophylactic antibiotics for incisional hernia repair has increased, particularly when using mesh[56,59,63,69]. In one study on factors affecting wound complications in ventral hernia repair[74], 91 per cent of patients undergoing mesh repair received prophylactic antibiotics compared with 71 per cent of those undergoing suture repair. However, the use of antibiotics did not appear to influence the incidence of wound complications. Still, the number of patients in each group was small and, in the absence of randomized clinical trials comparing results of repair with and without antibiotic prophylaxis, use of antibiotics in this context seems justified. First-generation and third-generation cephalosporins are most often used.

Hesselink and colleagues[75] found that the larger the hernia, the greater the risk of recurrence, but this was not corroborated by Luijendijk *et al.*[40], who found that size did not matter. However, the latter authors reported that prostatism in men, previous abdominal aortic aneurysm surgery and suture repair were all risk factors for recurrent hernia. Presumably prostatism is a risk factor because of the increased intra-abdominal pressure associated with straining at micturition. Other conditions such as chronic constipation[22], chronic obstructive airways disease[22], smoking[52] and occupational lifting[52], which may all cause increased strain on the repair, have also been implicated in recurrence. Koller and colleagues[51] found that the risk of recurrence was lower in women.

Conclusions

In spite of the relative paucity of randomized clinical trials, the larger studies published to date highlight unacceptably high rates of recurrence (up to 49 per cent) with open suture repair of incisional hernia. While there have been isolated smaller studies reporting lower recurrence rates using flaps of rectus sheath for the repair[19,23], a recent experts' meeting[9] agreed unanimously that both fascial coaptation and fascial overlap techniques should only be used for small incisional hernias.

The recurrence rates reported for open mesh repair have been consistently lower (0–10 per cent). This has been confirmed in four comparative studies[49–52] and a randomized clinical trial[40], which showed a significantly lower recurrence rate in the mesh group (46 *versus* 23 per cent) although complication rates did not differ between the groups.

Laparoscopic mesh repair produces similarly low recurrence rates (0–9 per cent) with acceptable morbidity. Five comparative studies[67,69–72], including a randomized clinical trial, showed advantages of laparoscopic over open mesh repair. Another study[68] showed no such advantage. The evidence available at present suggests that laparoscopic repair is feasible, safe and at least as effective as open mesh repair, although experience with the new method is still limited. As laparoscopic skills improve, it is likely that laparoscopic repair will become more widely performed, and this may make feasible a large multicentre randomized clinical trial comparing open and laparoscopic mesh repair. Even with the current level of evidence, encouraging training in the technique of laparoscopic mesh repair appears worthwhile. Some surgeons[69] feel that even now there is enough evidence to recommend laparoscopic repair as the first-line treatment for incisional hernia where this is available; where it is not, open mesh repair remains a suitable alternative.

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Pathogenesis of colonic diverticula

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Background: Diverticula are herniations through the colonic wall and are therefore likely to be due to a weakness within the wall, an alteration in intracolonic pressures or a combination of these factors. This article reviews these aspects in relation to the pathogenesis of colonic diverticula.
Methods: Medline and Science Citation Index searches were performed to locate English language articles relating to the pathogenesis of colonic diverticulosis published since 1960. Manual cross-referencing was also performed and some historical articles were included.
Results and conclusion: Several theories now exist about the development of colonic diverticula. The majority of the evidence suggests that the morphological changes are the response to a lifelong consumption of a low-residue diet. However, there are complex relations between colonic structure, motility and dietary factors, and it is likely that all of these (and possibly genetic influences) play a role in the pathogenesis to a greater or lesser degree.

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Introduction

Colonic diverticulosis is the most common structural abnormality of the colon. The exact incidence is not known but it is endemic in elderly populations of developed countries. Diverticula are herniations through the colonic wall and so are likely to be due either to a weakness in the colonic wall or to altered intracolonic pressure, or to a combination of these factors. The incidence continues to rise and changes in dietary patterns are thought to be responsible. However, the structural integrity and motility of the colon are influenced by a variety of factors and this article reviews these aspects in relation to the pathogenesis of colonic diverticulosis.

Search strategy

An internet-based search strategy of the Medline and Science Citation Index was performed using the keywords diverticulosis, diverticular disease, pathogenesis, motility, intracolonic pressure and structure in various combinations with the Boolean operators AND, OR and NOT. Only articles relating to the pathogenesis of colonic diverticula were considered. Human and animal studies were used and manual cross-referencing was also performed. Articles published in the English language between January 1960 and April 2001 were selected. Letters and most case reports were excluded, and abstracts are cited only when the full papers were unavailable.

Colonic motility

The complex nature of colonic motility[1] together with the relative inaccessibility of the organ make detailed descriptions difficult, although some generalizations are possible. The commonest motor pattern is tonic segmenting and rhythmic contraction, with the former creating stationary narrow rings that appear as the haustral markings. These segmental contractions normally serve to slow the faecal stream, thereby maintaining continence and permitting water absorption and electrolyte exchange. There are also infrequent propulsive peristaltic contractions that usually move in a caudal direction, occurring on average six times a day[2]. These high-amplitude propagated contractions are the manometric counterpart of the infrequent mass movements[3]. The myoelectrical activity leading to these movements and the alterations in pressure that occur as a result have both been implicated in the pathogenesis of colonic diverticulosis.

Intracolonic pressures

Painter *et al.*[4], using manometry and simultaneous cine-radiography, showed that the intermittent segmentation

Table 1 Summary of studies on intracolonic pressure

Reference	Year	Method	Main findings
Arfwidsson[5]	1964	Sigmoid manometry	Raised intracolonic pressure at rest and after food compared with controls. Excessive response following prostigmine
Painter and Truelove[6,7]	1964	Sigmoid manometry	Similar basal intracolonic pressures in controls and patients. Excessive rise in intracolonic pressure following prostigmine in the diverticular colon but no difference in pressure in normal segments of a diverticular colon
Painter *et al.*[4]	1965	Simultaneous radiography and sigmoid manometry	Segmentation within the colon leads to localized areas of raised intracolonic pressure
Attisha and Smith[8]	1969	Rectosigmoid manometry	Similar basal intracolonic pressures in controls and patients. Excessive rise in intracolonic pressure following prostigmine in the diverticular colon
Parks and Connell[9]	1969	Rectosigmoid manometry	Exaggerated colonic motility response to eating, detected with open-tipped catheters but not miniature balloons
Weinreich and Andersen[10]	1976	Sigmoid manometry	Significant correlation between colicky lower abdominal pain and high pressure activity following neostigmine. High pressure activity unrelated to the presence or absence of diverticula
Weinreich *et al.*[11]	1977	Barium enema and sigmoid manometry	Correlation between an increased haustral pattern and the presence of diverticula. No correlation between pressure activity and haustral pattern
Trotman and Misiewicz[12]	1988	Sigmoid manometry	Raised intracolonic pressure at rest and after food compared with controls
Cortesini and Pantalone[13]	1991	Sigmoid manometry	Significantly higher motility index in symptomatic diverticular disease than in asymptomatic disease and controls, both before and after food

process caused by the systematic contraction of the circular muscle divided the lumen into a series of compartments. They likened these to small bladders. The resulting raised intracolonic pressure within these compartments led to a substantial outward force on the colonic wall and these authors hypothesized that this was a prerequisite for diverticula formation. This temporal relationship was confirmed by Arfwidsson[5], who demonstrated a raised intracolonic pressure in patients in the prediverticular state, suggesting that the alteration in pressure was the primary abnormality. Since these pioneering works, several further studies have attempted to demonstrate alterations in intracolonic pressure objectively and relate these to changes within the colon (*Table 1*). Despite some conflicting conclusions, most agree that there is increased phasic pressure activity but that this relates more to symptoms than to the mere presence of diverticula. The inconsistencies that arise are probably the result of a number of factors. As Parks and Connell[9] have pointed out, confusion stems from methodological differences, in particular relating to bowel preparation and pressure sensors, as intracolonic pressure depends not only on the power of contractions but also on gut contents and outflow resistance. The heterogeneity of patients and disease states under investigation is compounded by poor patient selection[14], relatively small numbers, and differences in the anatomical sites under investigation. Furthermore, the period of measurement, which was often less than 2 h in one or two regions of the distal colon, is insufficient to detect the substantial pressure changes that may occur throughout the colon over a 24-h period as a result of as eating, emotion and defaecation. On these grounds it seems unreasonable to draw any firm conclusions from these investigations.

Myoelectrical activity

In addition to local pressure changes, a more generalized alteration in colonic motility has been implicated in the pathogenesis of diverticulosis. Extracellular electrodes show slow waves with spiking contractions corresponding to muscle contraction occurring at the peak of depolarization. It is believed that the slow waves originate from the interstitial cells of Cajal[15], as animals lacking these do not demonstrate slow waves[16]. Hiuzinga *et al.*[17,18] found that, *in vitro*, bursts of action potentials from diverticular specimens did not develop in a typical manner in response to cholinergic stimulation. This would favour segmental non-propagating contractile activity rather than propulsive motion normally associated with the action potentials.

In vivo studies have produced contradictory evidence, as slow-wave activity has been shown to be both unaltered[19,20] or possibly increased[20–23] in patients with diverticulosis. These discrepancies may relate to the difficulty in recording electrical activity from luminal electrodes with variable contact, especially in a deformed, fibrotic colon. Fast-wave activity is increased[24] in patients with diverticulosis and these changes have a tendency to persist following resection of the affected segment, suggesting an inherent alteration in electrical activity. However, the exact relevance of any myoelectrical changes in relation to mechanical events remains uncertain.

Structural aspects

From a structural perspective, the colonic wall is a composite of smooth muscle, connective tissue structures, and cellular and nerve elements embedded in an extracellular matrix. The mechanical properties of a complex structure such as this rely on the physical properties and durability of the individual elements and on their relative interactions.

Histologically, the normal colon consists of the mucosa, submucosa and the deeper muscle layers. The muscularis propria consists of an inner and an outer layer; the inner circular layer forms a continuous sheath around the bowel, whereas the outer longitudinal layer, although it does form a thinner circumferential layer[25], is concentrated mainly in three narrow bands (taeniae coli). The taeniae are variable in position, but in the sigmoid region one is situated in the mesenteric attachment and the other two are antimesenteric. Both muscle layers of the normal colon become progressively thicker distally as one moves toward the sigmoid, and in this region the taeniae become wider and closer together, eventually fusing in the rectosigmoid region. It is usual to find two rows of diverticula, one on either mesenteric side of the antimesenteric taeniae. In approximately half the cases, an additional row of diverticula is found between the antimesenteric taeniae[26].

The taeniae are approximately one-sixth shorter than the intestine to which they are attached and, as a result, the colon is compressed into haustra and sacculations, features of the bowel that allow large changes in the cross-sectional area of the lumen with comparatively little muscular effort; this permits propulsion of the faecal bolus[27]. Histologically, there is a close relationship and fascicular union between the taeniae and circular muscle. As a consequence, the taeniae act not only as shortening fibres for the intestine but also as longitudinal supports to which the circular muscle fibres are attached[28]. Thickening of the muscular layer is one of the most noteworthy and consistent features of diverticulosis, in both surgical[29] and post-mortem[30] specimens. This change is thought to predate the formation of diverticula[5] and has been implicated in the pathogenesis of diverticulosis by many authors[30–32].

Muscularis propria

The morphology and histological structure of the muscularis propria in diverticular disease has been studied frequently. In Arfwidsson's initial studies, he showed hypertrophy to be present in the circular muscle layer[5]. Although some of those subjects had previously experienced diverticulitis, similar specimens examined by Morson[29] showed no evidence of hypertrophy or hyperplasia.

Whiteway and Morson[31,33] also studied the histological structure of the muscularis propria in patients with uncomplicated diverticulosis, without changes induced by inflammation, obstruction or fibrosis. Although the muscle cells themselves were normal with no evidence of hyperplasia or hypertrophy (a finding supported by Slack[34]), both the circular muscle layer and taeniae coli were thickened, and the cause of this thickening was found to be different in the two layers. The taeniae were thickened secondary to elastin deposition, which also leads to contraction in this layer. There was an increase of over 200 per cent compared with controls[31]. As a result of the close fascicular union between the two muscle layers, contraction of the taeniae leads to lengthwise compression and thickening of the circular muscle layer, producing the characteristic concertina-like folds in the inner muscle layer. Between these folds the mucosa comes to lie in close apposition to the serosa, a situation that predates the formation of diverticula[35].

The elastin molecules are structurally formed in β-helical spirals which are laid down in a contracted state[36]. This retains the taeniae in the shortened position and gives them an almost cartilaginous texture. This can account for the gross contracture deformity seen in operative specimens[37] and leads to the inability of the colon to elongate normally when the circular muscles contract. It may also explain the increase in distensibility of the bowel wall that occurs in diverticular disease[9,38] and may be the reason why bran fails to alter the strength and tone of the colonic wall in affected patients[39]. Whiteway and Morson[33] suggested that the elastosis in the taeniae may be the initial change in diverticular disease, although mechanisms behind this are still unproven.

Leung and colleagues[40] showed that repeated intermittent distension *in vitro* could result in the increased synthesis of connective tissue components. It might therefore be hypothesized that the reduced faecal load produced by a Western diet, which distends the narrowed colon only intermittently, may initiate the deposition process in the intestine. This is supported by the finding that resection specimens show the presence of elastin fibres in various stages of maturity, suggesting that deposition occurs on a continual basis[33]. Interestingly, this argument may have validity as the pattern of elastosis present in diverticulosis is similar to that found in pulmonary hypertension, in which the pulmonary artery has to withstand increased pressure intermittently[41].

Smith[27], on the other hand, suggested that an alteration in diet has effects on the physical properties of the colon leading to the elastogenesis. The colon exhibits a mechanical property termed prestrain[42]. This phenomenon occurs in many living tissues that are held under varying amounts of stretch and may be demonstrated by the shortening of the colon or blood vessels when they are excised. Such a physical property allows maintenance of form as it permits tissues to expand in one direction without altering their length in another. Smith[27] suggested that the increase in elastin within the taeniae may be compensation for the reduction in prestrain secondary to a low-fibre diet. These observed changes within the taeniae may also be relevant from a motility perspective. The slow waves passing through the colon are generated from this part of the wall and their frequency has been shown to be altered in some patients with diverticular disease. Whether these two changes are related and whether the resulting alteration in slow-wave frequency is mechanically significant is, as yet, unclear.

Connective tissue

Within the normal colon, collagen and elastin form a widely spaced meshwork distributed throughout the wall, providing the major stress-bearing components with respect to intracolonic pressure. Whether changes in intracolonic pressure are present or not, connective tissue structural weakness would make the colon more susceptible to herniation of the more elastic mucosa through its wall. *In vivo* studies by Parks and Connell[9], and additional *in vitro* experiments by Smith *et al.*[38], showed an increase in compliance to be present in the diverticular colon, indicating a reduction in the structural integrity of the bowel wall. It is unclear whether the changes are localized or affect the whole colon. As both Smith *et al.*[38] and Parks[24] found these changes to persist in the remaining unaffected colon following resection, there may be a widespread reduction in structural integrity, leading to a general predisposition to form diverticula. These results also indicate that the compliance changes are not the product of the diverticula themselves.

However, the changes in collagen are likely to be qualitative rather than quantitative as collagen content remains unaltered in both muscle layers[31] and the whole colonic wall[43], compared with controls. The involvement of a possible genetic connective tissue defect is suggested by reports of diverticula in children and young adults with Marfans[44–46] or Ehlers–Danlos[47] syndrome. There is also a strong relationship between polycystic kidney disease and perforated diverticula[48]. Furthermore, while the area of genetic predisposition has yet to be fully explored, it is known that race is implicated in the pathogenesis of right-sided colonic diverticula, a condition more commonly seen in Far Eastern populations[49]. It may be that patients developing left-sided diverticula have a so far undetected genetic abnormality within their tissues, or are genetically more prone to the natural ageing process.

Mucosa

The majority of diverticula pass through the bowel wall at weak points in the circular muscle layer where the blood vessels penetrate it to supply the mucosa[50,51]; this suggests that pulsion may play a part in their pathogenesis. Colonic mucosa affords little tensile strength and is extremely expansile, a property that allows easy herniation through points of wall weakness. Historical articles[52] have suggested that an important aetiological factor is deposition of fat in the perivascular tissue, leading to an exaggeration of the potential space through which herniation can occur. However, modern studies have failed to show any preponderance of diverticulosis in obese subjects[53] and obesity is no longer recognized as an aetiological factor.

Ageing

Epidemiological evidence demonstrates an increase in the incidence of colonic diverticulosis with rising age; perhaps the most important finding in this respect is the reduction in tensile strength of the colonic wall with age[54,55]. The exact mechanism underlying this reduction remains uncertain. The major structural component of the submucosa is collagen[56], the fibres of which are arranged in a discontinuous mesh that, along with elastin, provides strength and expansibility and allows maintenance of shape[33,55]. Collagen fibrils demonstrate increased cross-linking with age[43,57] and, in the left colon, become smaller, more numerous and more tightly packed; this trend is more pronounced in diverticular disease[58]. Pace[59] demonstrated that the collagen tissue layer between the longitudinal and circular muscle in the normal colon is reduced with increasing age, although the individual fibres may increase in thickness up to the age of 60 years. The increase in thickness probably explains the constant collagen content within the colonic wall[43] and indicates that qualitative changes are of more importance than quantitative ones.

Elastin content increases throughout life in all layers of the colon[27], although its deposition predominates in the taeniae in patients with diverticulosis. The fibres become shorter, thicker and less wavy, which may account for the increase in distensibility of the diverticular colon[9,39]. There is also a steady increase in the thickness of the bowel wall with age, both longitudinal and circular muscle layers being affected. Consideration of this finding in relation to the mechanical studies indicating a reduction in tensile strength[54,55] suggests that the increase in muscle thickness with age is no compensation for the qualitative reduction in mechanical integrity of the colonic wall.

Dietary factors

Diverticulosis was described by Painter and Burkitt[60] as a disease of Western civilization; there is a large amount of historical and epidemiological evidence supporting the role of dietary factors in the pathogenesis of the condition[61,62]. In particular, diverticulosis is rarely seen in rural Africa or Asia, but becomes increasingly prevalent in people living in areas within these continents that have developed economically and acquired Western dietary habits. Populations moving from a rural to an urban environment experience an increased prevalence of diverticular disease, further supporting this hypothesis[63].

Diet dictates to a large degree the composition of luminal contents in the colon and this has implications for colonic structure and motility. It may also influence the chemical composition of the supporting structures, particularly the collagen fibres[64]. Large-bore colons are found in countries where a diet rich in fibre is consumed[65,66]. By contrast, the modern Western diet, which tends to be fibre depleted, produces less stool bulk, resulting in a colon of reduced diameter. The narrowed lumen, redundant mucosa and prominent muscular folds make the formation of the bladder-like compartments described by Painter *et al.*[4] during the segmentation process more likely, thereby raising the intracolonic pressure significantly and favouring the development of diverticula. Perhaps without these presumed physiological changes there would be a decrease in intracolonic pressure in a relaxed, empty intestine, leading to diarrhoea[67] as the reduced faecal stream would pass distally without resistance. The pressure required to distend the colon is greatest where the radius is small (law of Laplace) and so passage of faeces through a narrow colon requires greater intraluminal pressure. This suggests a greater stress in the wall of the narrow colon than would enhance the formation of pulsion diverticula.

There is also a positive correlation between transit time and the amount of fibre in the diet. Food residue generally passes through the African gut within 48 h, resulting in a bulkier stool without the need to strain[68]. By contrast, a typical English low-residue diet of the 1960s resulted in a transit time nearly twice as long[69], encouraging drying of the stool and increasing viscosity[65] and pressure generation. Ignoring the call to stool and flatus retention[70] also have a theoretical effect on intracolonic pressure, and have both been suggested as factors in the pathogenesis of diverticulosis, although convincing evidence in this area is lacking.

Animal studies

The above concept of the pathogenesis of colonic diverticulosis has been supported by work on animal models, the most comprehensive of which involved 1800 rats[71]. The demonstration of a significant relationship between dietary fibre, faecal output and transit time, and the observation of both single and multiple true acquired diverticula, validated the use of the rat as a model for colonic diverticulosis. Significant inverse relationships were demonstrated between the incidence of diverticula (and prediverticula) and the content of fibre within the diets. However, a major anatomical drawback is that rats have a complete layer of longitudinal muscle rather than the taeniae found in humans.

Rabbits have also been used in the study of diverticular disease because of their size and their similar colonic structure to that of humans. Hodgson[72,73] fed them a low-residue diet of white bread, butter, milk and sugar for 4 months, noting a general deterioration in condition but an increase in weight. Following stimulation with prostigmine, intertaenial diverticula appeared that mimicked the original description of Painter *et al.*[4]. In addition, the colonic motility index[74] increased by tenfold. The rabbit colons were thought to be contracted and the animals became constipated with a reduced faecal output (from 80 g to 3 g per day) and an increase in transit time (48 *versus* 24 h). Animal models have also permitted the effect of diet on the structure of the colonic wall to be examained. Wess *et al.*[64] found that a high-fibre diet fed to rats protected against collagen cross-linking and this was related to a decreased incidence of diverticula. Further work by this group[75] examined the hereditary aspect of the condition by assessing the effect of maternal diet on the incidence of diverticulosis. When newborn rats (regardless of maternal diet) were fed a low-fibre diet, they were found to develop diverticula. However, significantly fewer diverticula occurred in rats born to mothers previously fed a high-fibre diet. These studies appear to suggest that, in addition to current diet, maternal diet may also influence the development of colonic diverticula. Animal models of colonic inflammation[76,77] produce thickening of the muscular wall which may persist after resolution of the inflammation, suggesting that some of the muscular thickening noted in resection specimens of complicated disease may be secondary in nature.

Conclusion

Several theories exist on the development of colonic diverticula; much of the evidence suggests that the pathological changes are adaptive, resulting from a lifelong exposure to a low-residue diet. However, there are complex interactions between colonic structure, motility and diet, and it is feasible that all of these factors (and possibly genetic influences) play a role in the pathogenesis to a greater or lesser degree.

As the population ages, the increase in the incidence of diverticular disease is likely to continue and primary prevention with a high-fibre diet is probably not a feasible management option. Epidemiological evidence suggests that not only would such treatment need to be prolonged, but also the amount of fibre needed would be very high, much higher than in the typical Western 'high-fibre diet'. Although vegetarians have a lower incidence of diverticular disease[78], there is no good evidence that eating a high-fibre diet prevents the progression of established diverticulosis or reduces the incidence of complications.

Despite this, established treatment patterns involve mainly dietary manipulations, none of which is particularly effective[79]. Indeed, they may increase symptoms in a significant proportion of patients[80]. A possible explanation for such ineffectual treatment is that the reason for symptom generation is poorly understood. Furthermore, why certain patients with diverticula experience symptoms while others do not remains a mystery. It is worthy of note, however, that patients who have previously suffered bouts of diverticulitis are more likely to experience subsequent episodes of recurrent short-lived abdominal pain and altered bowel function[81,82]. This clinical situation seems analogous to the postinflammatory symptoms that are a recognized feature of other inflammatory conditions of the colon, such as infective enteritis[83] and inflammatory bowel disease[84].

A growing area of interest in the development of colonic symptoms such as these involves the effect of inflammation on the enteric nervous system in which complex neural circuits are central to normal gut function. A range of inflammatory insults in animal models has shown that even transient inflammation that leaves no residual scarring can profoundly alter bowel function[85,86]. Smooth muscle hypertrophy and increased sensitivity to abdominal distension have been demonstrated, possibly mediated via inflammatory cytokines[87,88], and structural changes in gut innervation have also been reported[77].

Human studies in this sphere are as yet limited, but an increased level of the neuropeptide substance P[89], which may relate to altered visceral sensation, has been demonstrated in patients with diverticular disease. Neuroproliferation within the appendix in association with an increased concentration of substance P and vasoactive intestinal polypeptide has been implicated in the pathophysiology of right iliac fossa pain in the absence of inflammation[90]. An alteration in the density of vanilloid receptors, which are found on enteric sensory nerves, has also been implicated in the pain and dysmotility of inflammatory bowel disease[91]. Limited studies of patients with diverticulitis have also demonstrated abnormal nerves with axonal sprouting suggestive of previous injury[92].

These findings are compatible with postinflammatory neural and muscle dysfunction and suggest a mechanism for the generation of chronic symptoms in diverticular disease. The actual number of patients with possible postinflammatory symptoms is likely to be considerable. It is estimated that 10–25 per cent of people with colonic diverticulosis will suffer inflammation at some point in their lives, with a recurrence rate of 7–35 per cent[93,94]. The process of inflammation, repair, neuronal plasticity and symptoms requires further evaluation as it is a sequence that is amenable at many stages to pharmacological manipulation. This offers potential future benefit to patients for whom at present there is little to offer.

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Review

Fracture of the penis

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Background: Sporadic reports of penile fracture give the impression of a rare trauma. The value of diagnostic investigations is doubtful and treatment options are controversial.

Methods: A Medline search from January 1966 to July 2001 using the terms 'fracture of penis', 'penile trauma' and 'coital injuries' was used to identify full texts of publications on fracture of the penis. Full texts of relevant references from these publications were also identified. Data extracted for review included authors, country and year of publication, number of cases in each report, aetiology, clinical features, investigations, treatment and outcome.

Results: In 183 publications 1331 cases were reported between January 1935 and July 2001. Most reports were from the Mediterranean region. The commonest causes were coitus and penile manipulations, especially masturbation. Most patients were in their fourth decade. Clinical features included sudden penile pain, detumescence, voiding difficulties, and penile swelling and deviation. Diagnosis was made mainly on clinical grounds. Associated injuries included urethral rupture. Predisposing factors included excessive force at coitus or manipulation, fibrosclerosis of the tunica albuginea and chronic urethritis. Most authors advocated early surgical repair using absorbable sutures. Complications of the injury included coital difficulty, urethral fistula, penile plaque and erectile dysfunction.

Conclusion: Penile fracture is not rare. Radiological investigations are expensive and may delay treatment. Current management favours early surgical exploration to prevent complications.

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Introduction

Fracture of the penis, *faux pas du coit*, is primarily a rupture of the corpus cavernosum that occurs when the penis is erect[1,2]. The rupture may extend to affect the corpus spongiosum and the urethra[3,4]. The first documented report of this fracture is properly credited to an Arab physician, Abul Kasem, in Cordoba over 1000 years ago[5], in spite of claims that it was first described in 1925[6,7]. The condition is under-reported[8,9] and is a urological emergency[8,10]. Victims are often reluctant, from shame, to come forward for treatment[11,12] and they may be reticent in their history[13,14]. The condition has also been observed in animals[15,16], and several authors assert that penile fracture in humans is rare[1,7,8]. The need for diagnostic investigations is doubtful and treatment options are controversial. A review of published cases of fracture of the penis in humans was undertaken to identify the incidence, clinical features, current investigations, treatment and complications of this embarrassing mishap.

Method

A Medline search from January 1966 to July 2001 using the terms 'fracture of penis', 'penile trauma' and 'coital injuries of penis' was carried out to identify publications reporting cases of fracture of the penis and its complications. Full texts of these publications from Medline and from other medical literature found in libraries visited were collected, together with full texts of relevant references found within these primary sources. Data extracted from these papers for analysis include authors, country and year of publication, number of cases in each report, presenting symptoms and signs of fracture, age of the patients, aetiology of fracture, investigations, associated injuries, treatment given, and complications of both the injury and its treatment.

Results

A total of 183 publications reporting cases of penile fracture were retrieved and studied; they included single and multiple cases. The largest series was reported by Zargooshi and contained 172 cases[17]. All except two[18,19] were retrospective studies and up to 1985 only 135 cases had been reported[5]. By June 1991, Mansi *et al.*[20] had identified 235 cases and reported an additional 14 cases of their own. Although Mydlo *et al.*[7] claimed that up to 1998 only 250 cases of penile fracture had been reported in the English language literature, in the 7 years between these two studies this present review was able to identify 517 published cases, showing an apparent rising incidence worldwide. It also identified over 1331 cases of penile fracture that appeared in the medical literature from January 1935 to July 2001. Some 231 cases reported in the Japanese literature in 1983[21] and a further 80 cases mentioned by Porst while commenting on the paper by Gontero *et al.*[18] were not included in this study because the publications were not available to the author. Thus about 1642 cases of penile fracture have been recorded from several countries (*Table 1*). Over half of the cases, 745 (56 per cent), originated in the Mediterranean Moslem region, including Turkey (*Fig. 1*).

Table 1 Countries reporting more than ten cases of penile fracture

Country	No. of cases
USA and Canada	250
Iran	240
Morocco	226
Turkey	117
Egypt	78
Saudi Arabia	44
UK	43
Japan	32
Bulgaria	31
Italy	30
Spain	22
China and Taiwan	21
Qatar	21
India	19
Germany	15
West Indies	14
Nigeria	11
France	11

Clinical features

Patients usually presented early in the acute state. Their ages ranged between 12 and 82 years (*Table 2*), with mean ages mostly in the fourth decade. Presenting complaints included a sudden cracking sound in the erect penis associated with pain and sudden detumescence[10,26,31]; a few patients continued with intercourse after the fracture[35,36]. Some described 'a "sharp cracking noise" in the penis also heard by [the] wife'[37] or partner[38,39]. The noise has been described as resembling 'the breaking of a corn stalk'[40] or 'the breaking of a glass rod'[13]. Other less common complaints were poor erection[26], postcoital meatal bleeding[22,24], haematuria[41], incapacitating erectile penile deviation[22,42], poor urinary stream[8] and urinary retention[2,42,43]. Findings at presentation included penile swelling, ecchymosis, penile deviation and a palpable gap or depression in the penile shaft[28,44,45]. The 'rolling sign' over a blood clot has been found useful in identifying the fracture site[17,46]. 'Egg plant deformity'[3,8,17], also called the 'aubergine

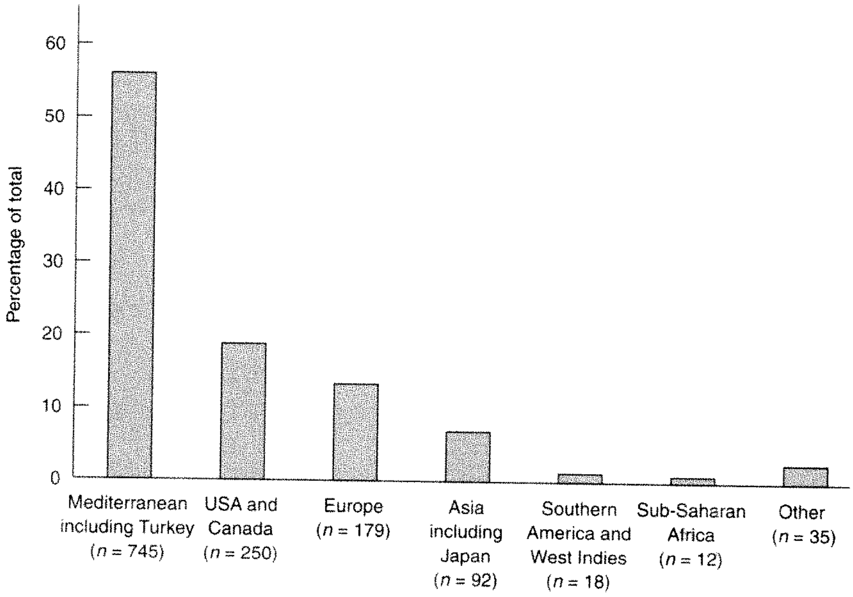

Fig. 1 Regional distribution of 1331 cases of penile fracture

sign'[47], consisting of swelling, bruising and deviation of the penis, has been used to describe the physical appearance of a fractured organ. A butterfly-shaped haematoma in the perineum may suggest a urethral injury[48]. Late presenters came with complications, such as erectile dysfunction presenting long after the predisposing trauma[26,49,50], penile deviation[20,27,29] and plaques resembling those of Peyronie's disease[1,22,51]. Other late presenting complications were urethrocavernous and urethrocutaneous fistula[32,52–54], as well as poor urinary stream (including urinary retention) due to urethral stricture[8,46].

Table 2 Number and age of patients with penile fracture (only those publications with complete column headings data are included)

Reference	Year	No. of cases	Age (years)*
Zargooshi[17]	2000	172	29 (12–80)
Asgari *et al.*[22]	1996	68	28 (14–50)
Mydlo[23]	2001	34	27 (18–38)
Uygur *et al.*[24]	1997	32	32 (18–52)
El Abd *et al.*[25]	1988	29	26 (18–61)
Hinev[26]	2000	25	35 (17–76)
Ozen *et al.*[27]	1986	25	33 (18–62)
Karadeniz *et al.*[10]	1996	21	33 (19–53)
el-Sherif *et al.*[1]	1991	21	35 (22–58)
Mansi *et al.*[20]	1993	14	32 (16–82)
Anselmo *et al.*[28]	1991	13	40 (23–51)
Tan *et al.*[29]	1991	12	42 (25–55)
Kuyumcuoglu *et al.*[30]	1990	12	32 (24–53)
Dincel *et al.*[31]	1998	11	27 (21–38)
Wang *et al.*[32]	1995	11	39 (23–60)
Al Saleh *et al.*[33]	1985	9	32 (25–50)
Fergany *et al.*[2]	1999	8	42 (24–60)
Mbonu and Aghaji[34]	1992	8	33 (21–50)

*Values are mean (range)

Aetiology

Fracture of the penis may occur 'by simple misfortune or as a result of sexual athleticism'[37,55]. The injuries that fracture the penis are generally regarded as blunt trauma[32,49,52]. The commonest cause relates largely to the geographical area of the report (*Table 3*). It is vigorous vaginal intercourse[2,26,28] in the Western hemisphere, where coitus is claimed to be the cause of injury in 30–50 per cent of cases[58]. Those from the Middle East[1,5,17,20] resulted mainly from penile manipulations at masturbation, including kneading the penis to achieve detumescence. Patients have fractured the penis in attempts to correct a congenital chordee[17] and to tuck an erect penis into underwear[41,50]. Rare causes include penile venous surgery[59]. Fracture has also been described following a fall from bed in sleep when the penis was erect[27,60], from disentangling the organ from a garment[61], while rolling over in bed during nocturnal tumescence[17,62] and spontaneously while urinating[29]. Reverse coitus, in which the woman lies on top of the man during sexual intercourse, has also been implicated in reports of acute penile injury[63–65], and brisk rotation of the woman at coitus may rupture a penile vein[66]. Penile fracture occurred during sexual intercourse in the standing position when the woman suddenly collapsed and fell down, causing an acute bending of the male organ[67,68].

Most coital fractures involved consensual heterosexual vaginal intercourse but one case occurred at an attempted rape of a female robbery victim[14]. An iatrogenic case

Table 3 Aetiology of penile fracture (only those publications with complete aetiological data are included)

Reference	Year	Country	Total no. of cases	Coitus	Manipulation
Al Saleh *et al.*[33]	1985	UAE	9	2 (22)	7 (78)
el-Sherif *et al.*[1]	1991	Qatar	21	2 (10)	19 (90)
Mansi *et al.*[20]	1993	Egypt	14	5 (36)	9 (64)
Asgari *et al.*[22]	1996	Iran	68	1 (1)	67 (99)
El Abd *et al.*[25]	1988	Saudi Arabia	29	4 (14)	16 (55)
Hinev[26]	2000	Bulgaria	25	18 (72)	4 (16)
Zargooshi[17]	2000	Iran	172	14 (8)	119 (69)
Karadeniz *et al.*[10]	1996	Turkey	21	21 (100)	0 (0)
Uygur *et al.*[24]	1997	Turkey	32	9 (28)	11 (34)
Nicolaisen *et al.*[41]	1983	USA	7	5 (71)	2 (29)
Wespes *et al.*[50]	1987	Belgium	7	3 (43)	1 (14)
Ruckle *et al.*[56]	1992	USA	9	3 (33)	2 (22)
Fergany *et al.*[2]	1999	USA	8	8 (100)	0 (0)
Bergner *et al.*[57]	1982	USA	5	3 (60)	2 (40)
Mbonu and Aghaji[34]	1992	Nigeria	8	5 (62)	3 (38)
Dincel *et al.*[31]	1998	Turkey	11	8 (73)	2 (18)

Values in parentheses are percentages

resulted during rectal surgery when the patient's penis was crushed between the two leaves of the operating table[12]. Some strange causes of penile fracture include 'banging an erect penis against a toilet seat' and 'being catapulted against the dashboard of a suddenly stopped automobile'[61], masturbating with the penis in the narrow neck of a cocktail shaker[69] as well as the penis being trapped in a car door[70,71]. Corpus cavernosal laceration of the flaccid penis by a direct blow[1,72], from the kick of a horse[73], a kick at football[1] and by gunshot wounds[8,74] have been reported. There are no reports of the injury occurring from anal intercourse or fellatio. The influence of penovaginal disproportion has been mentioned anecdotally[12].

Investigations

Most authors agree that the diagnosis of penile fracture is largely clinical[17,18,26]. However, several radiological investigations, including cavernosography[7,10,75], urethrography[27,53,76], ultrasonography[26,77,78], magnetic resonance imaging[79,80], colour Doppler duplex scanning[81] and angiography[49,50], have been carried out. Urethroscopy[46,47,82] and diagnostic exploration[18,39,83] have also been employed in the investigation of suspected penile fractures.

Pathology

A penile fracture involves disruption of the tunica albuginea and the enclosed corpus carvenosum, which may extend to the corpus spongiosum and urethra[3,4,84]. Following the fracture, a haematoma forms which, if confined within Buck's fascia, is limited to the penile shaft. When the fascia is disrupted, ecchymosis spreads to the scrotum, the perineum and the suprapubic area[42,44]. Blunt trauma at coitus or penile manipulation may damage the erectile tissue of the penis without injury to the tunica albuginea[37].

Rupture of the tunica albuginea is usually unilateral and transverse[22,24,31,45,51]. Longitudinal tears are rare[65,79], occurring as an extension of a transverse tear[51]. Bilateral corporal tears also occur[7,39,71]. Rarely, both corpora cavernosa and the urethra may rupture simultaneously[3,84]. The fracture occurs more often in the proximal shaft[2,17,51] and is located ventrally in coital injuries[79]. Fractures have also been reported in the distal third of the penis[46,79]. Often reports that lateralized the fracture, the right side was more often affected than the left (in nine[5,10,17,30–32,34,51,75]), and an even distribution was reported in one[50].

Associated or mimicking injuries included urethral rupture[4,8,63] and penile vascular injury, such as rupture of the penile superficial dorsal vein[66], deep dorsal vein[83,85,86], dorsal artery[87] and deep cavernous artery[49]. Others are Mondor's disease (thrombosis of the superficial dorsal vein)[38,88] and lymphangitis of the penis[89,90]. Urethral rupture more commonly occurs in association with a bilateral corpus cavernosal tear than unilateral tear[2,3,53]; up to 38 per cent of penile fractures are associated with urethral rupture[2,53,91]. However, the frequency of urethral involvement ranges between 0 and 3 per cent in the Persian Gulf and Japan[1,17,22], where the main aetiology of fracture is non-coital penile manipulation. Rupture of the suspensory ligament of the penis, which results in penile dislocation[92,93] and the 'flail' penis[37], may occur acutely during coitus.

In a recent study of six patients with coital penile fracture[51], fibrosclerosis of the tunica albuginea and chronic cell infiltrates were found in five. Periurethral inflammation, as occurs in gonococcal urethritis, has been aetiologically implicated in penile fracture[13,34,61].

Treatment

Treatment may be either conservative or surgical. Conservative measures include cold compress, anti-inflammatory agents and fibrinolytics, such as streptokinase and trypsin[42,71,73]. Antimicrobial agents are recommended by some authors[2,17,24]. Antiandrogens such as stilboestrol[25,60,94] and sedatives such as diazepam[20,26] have been used to prevent erection in the convalescence period. Conservative management has been advocated when cavernosography suggests a mild laceration of the tunica albuginea[55,57] or an intact tunica in spite of a history strongly suggestive of a penile fracture[44].

Urgent surgical repair, however, first done in 1935[61] and later recommended in 1957 by Fernström[12], is presently overwhelmingly favoured for the treatment of penile fracture[2,17,26,28,41]. It includes evacuation of haematoma and ligation of vessels[8,85,95], debridement and suture of lacerations of the tunica albuginea[42,86], urethral stenting[3,42], end-to-end urethral anastomosis[50,51], vascular anastomosis[96] and insertion of dermal grafts[82,97] or a prosthesis in erectile dysfunction to restore erectile ability[51,98]. Surgery may also be carried out for deformity and coital difficulty after initial healing has occurred[12,67,97]. The repair has been performed under local anaesthesia[68,91,99], spinal anaesthesia[2,73,100] and general anaesthesia. Options for access to repair a penile fracture include these incisions: (1) degloving (also called circumcising or subcoronal)[17,23,24,58]; (2) direct longitudinal[5,20,27,76]; (3) inguinoscrotal[64,74]; (4) high scrotal midline raphe[101]; and (5) suprapubic[96]. Closure of the tear in the tunica albuginea is performed longitudinally along the axis of the shaft[10,56]. Absorbable[20,24,58] and non-absorbable[17,31,45] sutures have been used; non-absorbable with the knot buried in the

Table 4 Complications of penile fracture

- Penile deviation[22,27,29]
- Penile pain at intercourse[24]
- Painful erection[27,36]
- Pseudo-diverticulum[36,104]
- Erectile dysfunction[26,78,105]
- Penile aneurysm[26]
- High-flow priapism[106,107]
- Recurrence or penile refracture[17,45,56]
- Lymphoedema from disruption of the lymphatics[74]
- Annular restriction[51]
- Penile abscess[39,40,108]
- Penile skin necrosis[57,76]
- Penile amputation for penile gangrene[109]
- Penile plaques[22,37,51]
- Fistula
  - Urethrocavernous[3,54,72]
  - Spongiocavernous[82]
  - Urethrocutaneous[32]
- Arteriovenous fistula[50]
- Urethral stricture[6,8,46,110]
- Psychiatric disturbance[67,78,111]

tunica albuginea or corpus cavernosum has been recommended for recurrent cases[102]. Urethral catheters have been used as stents[5,11,28], to protect the repair by urinary diversion[2,26,58], and to avoid inadvertent injury to the urethra during the repair of a fracture[24,31,82]. Some authors applied drainage at the site of surgery[8,27,75]. Gentle-pressure dressings[41,103] may be applied with any form of treatment.

Complications

Complications (*Table 4*) may occur with or without either form of treatment. There is an increased duration of hospital stay in the conservatively treated group and the results of delayed surgery are poorer than those of early operation[26,41]. Pseudo-diverticulum[36] results from deficient scar formation allowing the corpus cavernosum to distend during erection[104]. High-flow priapism manifests as painless persistent erection without sexual desire[107]. Penile refracture at coitus has been reported at 3 weeks after the initial injury[56] and, while it is often ipsilateral[45,102], contralateral recurrence is recognized[56]. Abstinence from coitus and penile manipulations for 6–8 weeks after a fracture has been recommended to prevent recurrence[2,56,102].

Discussion

The microarchitecture of the penis consists of a combination of transverse collagen fibres and longitudinally aligned elastic fibres. The latter prevent overstretching of the former at erection and enable their return to the resting position at detumescence[112]. The tunica albuginea, tightly applied to the erectile corpus cavernosum, is among the toughest of the body's fascias. It measures about 2 mm in thickness, thinning to only about 0·25 mm during erection[79,113]. At this thinness, the tunica becomes vulnerable to rupture during sudden acute bending[55,103] such as may happen when the erect penis slips out of the vagina and is thrust against the perineum or symphysis pubis[20,26,41]. It may also occur during penile manipulation; in erection, the penis loses much of its pliability and mobility of the flaccid state[3,53,74]. 'Erection converts the safe, flaccid penis into a vulnerable organ'[53]. Furthermore, during coitus, the dorsal penile vessels, engorged and interposed between the hard corpora cavernosa and the bony structures of the vagina, are prone to traumatic rupture[48].

Rupture of the tunica albuginea, and subsequently of the corpus cavernosum at erection, occurs when the intracavernous pressure exceeds 1500 mmHg[51]. Some authors[2,20,26,42] recommend that the term 'fracture' should be restricted to rupture of the penis when it is erect. While the flaccid penis is pliable, the erect penis is rigid enough and its tunica albuginea inelastic and thin enough to snap at trauma, breaking (or 'fracturing') the enclosed engorged rigid corpus cavernosum in a manner similar to the fracture of bone. Contrary to the suggestion that 'fracture of the penis' is a misnomer[114], the word 'fracture' is not restricted to bone[115]. It has been advocated that penile injury from a sporting kick, as well as from bites of animals and humans resulting in laceration of the corpora in a flaccid penis, should not be regarded as 'fracture of the penis'[29,94], although the treatment is similar. Also, rupture of the suspensory ligament of the erect penis should not be regarded as fracture of the penis[85,91,94] because of the differing clinical features and treatments of the two conditions. Furthermore, fracture of the penis affects the erectile tissues and rupture of the suspensory ligament is not followed by detumescence.

Clinical features

Believing that the presumed predisposing pathology, fibrosclerosis, is rare, De Rose *et al.*[51] stated: 'Therefore, one can understand how only small minorities of people are at risk of penile rupture even if the traumatic impact of the erect penis to the partner's pelvis is a quite common event during sexual intercourse'. Despite under-reporting of fracture of the penis, more than 1642 cases have been published in the literature over a 65-year period; the incidence appears to be rising and the condition is certainly not as rare as has been claimed by some. The apparent rising incidence may even increase with the introduction of effective treatments for erectile dysfunction, such as sildenafil.

The clinical features of a typical penile fracture may occur without a true fracture when there is an intracavernous or extratunical haematoma without a tear in the tunica albuginea[79,85]. While some authors consider that the features of isolated penile vascular injury and penile fracture are similar[10,83,103], detumescence occurs more slowly in vascular rupture[85]. Isolated urethral rupture differs from penile fracture by the absence of a snapping sound, penile deformity or palpable penile defect[63]. Although urethral bleeding (urethrorrhagia)[26], haematuria and difficulty with voiding[2,6] indicate a urethral rupture, the absence of these features does not exclude an associated urethral injury[6,7]. Furthermore, there may be no urethral rupture in spite of postcoital urethral bleeding[7,22,24]. The present study suggests that penile fracture occurs mainly in patients between 30 and 40 years of age (*Table 2*). The preponderance of mishap in this relatively young age group may reflect higher frequency and greater vigour of sexual intercourse. Also, the pressure of tumescence in youth, perhaps aided by healthy vascular channels, may contribute to the vulnerability of the younger penis to fracture.

Aetiology

Many of the reported cases of penile fracture (56 per cent) occurred in the Mediterranean region (including Turkey); this was observed by Esterlit *et al.*[86]. Penile manipulation, as in masturbation, is a major cause of fracture of the erect penis in this region, while vaginal intercourse is the commonest cause in the Western hemisphere. One might speculate that the difference in aetiology between these two regions may be explained by the strict prohibition of extramarital sexual intercourse in Moslem areas[11,22]. The higher incidence of penile fracture in the Mediterranean region may be because it takes longer to obtain orgasm by penile manipulation than by coitus. Alternatively, there may be a much higher prevalence of sexual intercourse in the Mediterranean region compared with other regions. The preponderance of coital cases from certain countries, including the USA, perhaps reflects a more permissive cultural attitude to coitus. Occasionally, the history from the patient with a penile fracture may be misleading[14,57]. The veracity of such claims as fracture from stroking the erect penis[10,22], spontaneously during sleep[27,60] or at micturition[29] is difficult to substantiate.

Investigations

Although the diagnosis of a penile fracture is largely clinical, cavernosography and urethrography have been strongly advocated by some authors. Cavernosography is easy to perform on the operating table, with or without anaesthesia[42,116]. Porst, in the commentary on the study by Gontero *et al.*[18], recommends the procedure for evaluating patients presenting after 24 h of injury, in order to select conservative or surgical treatment. However, cavernosography is an invasive procedure and is associated with complications such as priapism[26,82,116], as well as allergic reactions[116] that may lead to fibrosis of the corpus cavernosum[42]. Furthermore, false-negative results[7,44] have been encountered and the procedure cannot identify significant traumatic vascular lesions[83].

Urethrography too has limitations and may not reveal a urethral rupture in spite of postcoital urethral bleeding[22,31,41]. Ultrasonography, also called echotomography[26], is the cheapest investigative procedure in penile fracture. However, it is difficult to interpret in the presence of 'pronounced subcutaneous blood embedding and oedema'[82]. While magnetic resonance imaging is the most accurate investigative procedure and may help to determine the need for surgical intervention, the cost is substantial and it imposes unnecessary delay in management[26,80]. The efficacy of colour duplex ultrasonography[81] requires further evaluation. Angiography is indicated in the investigation of erectile dysfunction[49,50] but cannot be recommended in the acute situation of a penile fracture. Diagnostic exploration is warranted in appropriate clinical circumstances when cavernosography is negative[18,39,83], but even this may occasionally fail to reveal a corporal tear[26].

Pathology

Rupture of the corpus cavernosum has been regarded as a vascular complication of sexual intercourse[48] because of its specialized vascular anatomy. Specific arterial and venous lesions have been described[47,66] which may result in erectile dysfunction several years after an injury[18,50,83]. Post-traumatic penile lymphangitis and Mondor's disease of the penis, both of which may be indistinguishable clinically[117], are rare and usually appear late after an initiating trauma.

With the finding of fibrosclerosis of the tunica albuginea as well as chronic cell infiltrates in patients with coital penile fractures, De Rose *et al.*[51] concluded that 'structural anomalies could alter the mechanical properties of the tunica albuginea, representing a weakening factor of the corpora cavernosa and thus a predisposing factor for traumatic rupture of the erect penis' from blunt trauma. Structural abnormalities of the tunica albuginea, as well as the effect of periurethral infection and fibrosis from gonococcal infection, may lead to some loss of its elasticity[34,51,61], predisposing to corporeal and urethral rupture. While penovaginal disproportion has been implicated as a factor in coital vaginal injury[118,119], its influence in penile fracture cannot be determined from the reports reviewed here. It is curious that there has been no reported concomitant injury in a sexual partner of a patient with a penile fracture.

It may be of therapeutic use to classify penile fracture as 'simple' or 'compound', simple fractures being those in which the skin and urethra are intact, while compound fractures are associated with urethral rupture. In bilateral coital cavernosal fracture associated with urethral rupture, the corpus spongiosum may also be ruptured to cause a tricorporeal fracture. The preponderance of fractures at the proximal shaft[2,17] may be explained by the proximal location of the fulcrum of the erect penis[11]. The preponderance of the lesions on the right side is interesting, but cannot be explained from available data. However, coital posture and a postulated right-sided penile anatomical weakness have been suggested[34].

Urethral injury has been reported more frequently in association with coital fracture than from manipulation injury. This may be explained by postulating that more vigorous force is expended in coitus, compared with the prolonged but lesser force involved in masturbation[2,17,22]. Urethral rupture may also occur without corpus cavernosum laceration[58,63,64]. It is difficult to ascertain whether the alleged predisposition of reverse coitus to urethral laceration[32,63] is related to an increased force of coitus in this position. However, while penile fracture from reverse coitus was associated with urethral rupture in some reports[32,63,120], the urethra was unaffected in others[63,64,73].

Treatment

The treatment of penile fracture has undergone an evolution. It used to be held that 'conservatism is the watchword concerning the care of these cases'[13]. Conservative treatment is now the exception rather than the rule, as its results are less satisfactory than those of emergency surgical repair. Philp and Collin[121] simplified the criteria for the option of conservative treatment by recommending it for those without penile deformity, while advocating operation for those with deformity. Conservative treatment has also been recommended when the cavernosal bodies are intact, but this may be problematic as it is difficult to exclude cavernosal rupture completely in the acute penis, even with expensive and time-consuming radiological procedures[7,26]. Although several authors have used antibiotics empirically, uncomplicated penile fracture probably does not require this[1,41,86]. Nevertheless, when the urethra is ruptured, resulting in a 'compound' fracture, antimicrobial drugs are indicated, although wound infection may still occur[31]. The use of sedatives and oestrogens to suppress erection during convalescence is probably unnecessary[29], especially as the dose required to prevent erection is likely to produce undesirable somnolence[94]. Furthermore, it has been suggested that, rather than abolish postfracture erection, its presence, while not putting the repair at risk of disruption, reduces the patient's anxiety about impotence and may have an overall beneficial psychological effect[1].

Two reviews of the results of conservative *versus* surgical treatment[41,103] indicate the general superiority of the latter. Operation can be done as a day case under local anaesthesia[68,99] with the added benefit of reduced hospital stay and overall cost of treatment. However, in a study by el-Sherif and associates[1], patients treated conservatively spent an average of 2·3 days in hospital while those treated surgically spent 4·1 days. These authors explained that patients in the conservative group were not catheterized and were not taking medication that required inpatient care, but different endpoints were applied to the two groups.

While venous injury has been treated successfully by ligation[66,83,85], the treatment of arterial injury is controversial, the options being ligation[87], arterialization of the dorsal vein[122] and microsurgical arterial anastomosis[96]. The results at present are unsatisfactory[49]. It has been suggested that weak erection after a simple repair of a penile fracture is usually due to a venous leak into the corpus cavernosum rather than to an arteriogenic pathology; if this is correct, the need for microsurgical arterial repair is obviated[18].

The choice of access to repair a penile fracture is probably a matter of custom or preference. A degloving incision allows excellent exposure of the three corpora[24,123] but may be complicated by abscess and skin necrosis[20,75]. A direct longitudinal incision over the presumed site of fracture is simple, but may be associated with poor cosmesis[76]. Inguinoscrotal incision has been advocated for proximal fracture and for those occasions when penile oedema is marked enough to threaten skin viability[74]; it avoids the lymphoedema that often complicates a degloving incision but wound infection may result in an ugly scar as well as penile angulation. In addition, urethral repair is difficult using this access. A high scrotal midline raphe incision avoids the excessive dissection of the degloving incision and gives good cosmesis. It can also be a route for a proximal degloving procedure[101]. A suprapubic incision[96] gives access to the three corporeal bodies and is useful for penile vascular surgery. The multiplicity of access options for the repair of penile fracture suggests that none is ideal for all situations.

In view of the high incidence of urethral laceration associated with bilateral corporeal ventral penile fracture, Fergany *et al.*[2] advocate exploration of the corpus spongiosum in this situation. They also suggest exploration of the other corpus cavernosum when one is fractured and the urethra is simultaneously ruptured. While a laceration in the tunica albuginea is usually sutured longitudinally in the axis of the penile shaft, closure should be carried out transversely if narrowing will result from a longitudinal closure[10,56]. The need for catheterization should be cautiously appraised to reduce the risk of infection and further trauma to the urethra[1]. The same caution should be applied to the use of drains. Dressings applied in any form of treatment of a penile fracture should be loose, exposing the glans, in order to avoid or detect ischaemia early on[3,103]. The integrity of a surgical repair as well as the state of the other corpora can be assessed on the operating table by intracavernous injection of isotonic saline to produce artificial tumescence[23,101].

Complications

Complications of penile fracture (*Table 4*) occur in both conservatively treated and operated patients, but they are commoner in the former. Still, a recent report of five young men clinically suspected of having a penile fracture who refused diagnostic investigation and surgical treatment documented no significant complication after a 12-month follow-up[19]. Some complications of penile fracture, such as penile deviation due to fibrosis at the site of trauma[22,28], penile pain at intercourse[24], painful erection[27,36] and erectile dysfunction[26,49], are also cardinal features of Peyronie's disease. This may lend credence to the trauma theory of the aetiology of Peyronie's disease[124]. It has been suggested by some that the fibrous plaque that occasionally occurs at the site of the laceration of the corpus cavernosum causing penile deviation[22,29] should not be confused with Peyronie's disease, which is postulated to be a systemic desmoplastic condition[53].

Vascular injury may present late with erectile dysfunction, by which time any treatment tends to yield poor results[49]. In addition to abscess complicating a fractured penis[37,40], the necrosis that occurs in some conservatively managed cases[57,76,109] is probably a feature of Fournier's gangrene.

Prevention

Prevention of penile fracture requires knowledge of the predisposing factors. While no controlled study has been done, suggested predisposing factors amenable to preventive measures include vigorous sexual intercourse, forceful penile manipulations, and unusual coital positions and postures, such as reverse coitus. The recommendation of the use of non-absorbable suture materials to repair recurrent penile fracture[45,102] suggests that such suture should also be used in the initial primary repair[102], although residual postoperative fibrosis may be associated with its use[31]. Spongiocavernous fistula[82] may be avoided by separating the spongiosum from the cavernosum during repair. Although several authors advocate abstinence from coitus and penile manipulation for 6–8 weeks after an episode of penile fracture to prevent refracture, Uygur *et al.*[24] reported no recurrence after up to 13 years' follow-up in 32 patients, some of who resumed sexual activity within 2 weeks of treatment. Also, abstinence would not prevent penile fracture that occurs during unconscious nocturnal tumescence.

Conclusion

Fracture of the penis is under-reported by patients and clinicians[5,9,40] and, apart from one report[55], it is invariably claimed to be rare. This situation may reflect a sociocultural inhibition in patients and/or apathy in clinicians. Some of the less severe injuries may be responsible for late presentation with penile deviation due to fibrosis at the trauma site. Coitus and penile manipulations are the commonest causes of penile fracture. While the condition can be diagnosed clinically without radiological investigation, associated injuries, especially vascular ones, require careful consideration and appropriate radiological assessment. The modern consensus overwhelmingly favours early operation rather than conservative management. Abstinence from coitus and from penile manipulation remains the only reliable means of prevention of these injuries, but this is hardly a pragmatic proposition.

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Original article

Levels of the chemokines growth-related oncogene α and epithelial neutrophil-activating protein 78 are raised in patients with severe acute pancreatitis

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Background: Multiple organ dysfunction syndrome secondary to systemic leucocyte activation is the major cause of death following an attack of acute pancreatitis. Although plasma levels of interleukin (IL) 8 are known to be raised in acute pancreatitis, levels of other CXC chemokines such as growth-related oncogene (GRO) α and epithelial neutrophil-activating protein (ENA) 78, which are also potent neutrophil chemoattractants and activators, have not been measured.

Methods: Timed plasma samples were obtained from 51 patients with acute pancreatitis, 27 with a severe attack and 24 with mild disease according to the Atlanta classification. Samples were analysed to determine levels of C-reactive protein (CRP), IL-8, GRO-α and ENA-78.

Results: Plasma levels of IL-8, GRO-α and ENA-78 were increased in patients with severe as opposed to mild acute pancreatitis as early as 24 h following disease onset. Using cut-off levels of 7 pg/ml for IL-8, 70 pg/ml for GRO-α and 930 pg/ml for ENA-78, peak levels within the first 24 h of admission had an accuracy of 81, 71 and 87 per cent respectively in predicting the severity of an attack of acute pancreatitis.

Conclusion: In patients with severe acute pancreatitis plasma levels of GRO-α and ENA-78 were raised in addition to those of IL-8, suggesting that all three chemokines are involved in the inflammatory response in this condition.

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Introduction

Acute pancreatitis is common; the incidence in the UK is about 40 cases per 100 000 population, and is increasing[1]. In the majority of patients the condition is mild, but about 25 per cent of patients suffer a severe attack and up to half of these will die[2]. Most deaths occur during the first week[1,3] and are secondary to a massive systemic inflammatory response syndrome (SIRS) with consequent multiple organ dysfunction syndrome (MODS). Activated leucocytes, particularly neutrophils, within the circulation and in organs such as the lung mediate organ damage[4,5].

Leucocyte activation and migration into tissue is a highly regulated process[6]. Chemokines (chemotactic cytokines) are a family of small inducible cytokines (8–12 kDa) that function as effector molecules in directing leucocyte trafficking in the initial stages of inflammation[6,7]. They can be broadly subdivided on a structural basis into the CXC subfamily, in which the first two of four conserved cysteine residues are separated by another amino acid, and the CC subfamily, in which the first two cysteine residues are adjacent. CXC chemokines can be further subdivided into two groups, those containing the conserved amino acid sequence glutamic acid–leucine–arginine (ELR) immediately preceding the first cysteine residue at the *N*-terminus and those lacking this motif. The structural classification of the chemokines also determines their biological activity. CXC chemokines possessing the

ELR motif include growth-related oncogene (GRO) α, epithelial neutrophil-activating protein (ENA) 78 and interleukin (IL) 8, and all are potent neutrophil chemoattractants and activators; those lacking the motif have little, if any, neutrophil-activating activity[7].

Interaction of CXC chemokines with neutrophils occurs through two main cell surface receptors: CXC receptor 1, which binds IL-8 only, and CXC receptor 2, which binds GRO-α and ENA-78 in addition to IL-8[8]. The functional consequences of the different receptor binding profile are not yet clear, although the CXC receptor 2 may be downregulated in severe sepsis[9]. ENA-78[10] and GRO-α[11], like IL-8, induce neutrophil chemotaxis, degranulation and an increase in intracellular calcium concentration.

IL-8 is the best characterized of the ELR-positive CXC chemokines and is implicated in the regulation of neutrophil migration and activation in many different inflammatory diseases[7]. Plasma levels of IL-8 are raised in patients who suffer a severe attack of acute pancreatitis[12–16]. GRO-α and ENA-78 levels are increased in bronchial lavage fluid from patients with adult respiratory distress syndrome and in urine from patients with urogenital sepsis[17–19], while ENA-78 levels are raised in synovial fluid taken from patients with rheumatoid arthritis[20]. Studies addressing the role of either GRO-α or ENA-78 in acute pancreatitis are lacking.

Patients and methods

Study design

Fifty-one patients with an attack of acute pancreatitis who presented to the Royal Liverpool University Hospital between March 1997 and March 1999 were recruited within 48 h of hospital admission. Acute pancreatitis was defined as acute abdominal pain with a typical clinical picture and a serum amylase level of at least three times the upper limit of normal. The time of onset of symptoms was recorded at recruitment. The Glasgow score[21] was determined 48 h after admission and the Acute Physiology And Chronic Health Evaluation (APACHE) II score[22] at the time of recruitment. A severe attack was defined as the development of local or systemic complications according to the Atlanta classification[23]. Patients with a history of acute pancreatitis within 6 weeks of the current admission, pre-existing chronic pancreatitis or known neoplastic disease were excluded. Also recruited were a further 25 healthy volunteers to determine baseline plasma chemokine levels. This study was approved by the local research ethics committee in Liverpool.

Sample collection

Blood was taken at the time of recruitment, 12 h later and daily until day 7 or discharge from hospital. Samples were collected in potassium ethylenediamine tetra-acetic acid and centrifuged within 3 h of collection at 3000*g* for 15 min at 4°C. Plasma was then aliquoted and stored at −70°C for later analysis.

C-reactive protein

Plasma C-reactive protein (CRP) levels were measured by an immunoturbidimetric assay (Boehringer Mannheim, Lewes, UK) using an automated analyser (Hitachi 747; Hitachi, Maidenhead, UK). The reference range was lower than 5 mg/l, with intra-assay and interassay variations of less than 5 per cent.

Chemokines

Plasma levels of IL-8, ENA-78 and GRO-α were determined using a sandwich enzyme-linked immunosorbent assay. Matched antibody pairs and recombinant protein, i.e. monoclonal antichemokine antibodies, biotinylated monoclonal antichemokine antibodies and recombinant proteins, were purchased from R & D Systems (Abingdon, UK).

Immulon II microtitre plates (Dynex Technology, Ashford, UK) were coated with purified antichemokine capture antibody in disodium phosphate 0·1 mol/l (pH 9·0) and incubated for 16 h at 4°C. Plates were washed with phosphate-buffered saline (PBS)/Tween-20 (pH 7·0). Non-specific binding was blocked by addition of PBS containing 5 per cent bovine serum albumin (BSA) for 30 min and the plates were washed again. Standard recombinant chemokines in the range 0–2000 pg/ml and plasma samples were diluted in PBS containing 5 per cent BSA and 0·05 per cent Tween-20, then added to wells and incubated for 2 h at room temperature. The plates were washed before the addition of biotinylated antichemokine detection antibody and incubated at room temperature for 1 h. After washing, a solution containing horseradish peroxidase avidin-D 5 mg/ml (Vector Laboratories, Peterborough, UK) in PBS, 5 per cent BSA and 0·05 per cent Tween-20 was added and incubated for 30 min at room temperature. Finally, plates were washed before the addition of the substrate 3,3′,5,5′-tetramethylbenzidine. Colour development was monitored at 450 nm with a spectrophotometer (Anthos Reader 2001; Anthos Labtec

Instruments, Salzburg, Austria). The reaction was stopped with sulphuric acid 0·18 mol/l and optical density was measured at 450/690 nm. Plasma chemokine levels were then derived from the linear region of the standard curve. The intra-assay variation was less than 5 per cent in assays for all three chemokines. Assays were performed over 18 months beginning in October 1998. At the beginning of this interval spiked plasma samples were aliquoted and stored at −70°C. These aliquots were included in each assay. The interassay variation calculated using these samples was 11 per cent for IL-8, 6 per cent for ENA-78 and 6 per cent for GRO-α.

Table 1 Characteristics of patients with acute pancreatitis and normal controls

	Pancreatitis		
	Mild	Severe	Healthy controls
No. of patients	24	27	25
Sex ratio (M : F)	10 : 14	15 : 12	11 : 14
Age (years)*	67·5 (24–88)	68 (40–90)	41 (23–82)
APACHE II score*†	6·5 (0–11)	11 (3–27)§	
Glasgow score*‡	2 (0–5)	4 (0–7)§	
Inpatient stay (days)*	7·5 (4–21)	15 (7–76)§	
Aetiology			
Gallstones	15	19	
Alcohol	2	3	
Idiopathic	6	5	
Carcinoma of pancreas	1	0	

*Values are median (range). †At recruitment; ‡48 h after admission to hospital. APACHE, Acute Physiology And Chronic Health Evaluation. §$P < 0·01$ *versus* mild acute pancreatitis (Mann–Whitney *U* test)

Statistical analysis

Results in the text are presented as median (range). The Kruskal–Wallis and Mann–Whitney *U* tests were used for comparisons. Receiver–operator characteristic analysis was used to choose the best cut-off values for diagnostic indices using peak plasma levels 48 h after hospital admission. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy for predicting severe pancreatitis were then calculated at the various timepoints[24]. Significance was set at $P < 0·05$.

Results

Twenty-seven patients suffered a severe and 24 a mild attack of acute pancreatitis (*Table 1*). The complications that

Table 2 Peak plasma levels of C-reactive protein, interleukin 8, growth-related oncogene α and epithelial neutrophil-activating protein 78 24 and 48 h after symptom onset, and 24 and 48 h after hospital admission, for the prediction of severity in acute pancreatitis

	Mild	Severe	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
24 h after symptom onset								
CRP (mg/l)	20 (2·5–71)	89 (11–334)†	> 150	30	100	100	61	66
IL-8 (pg/ml)	0 (0–5)	31 (0–180)†	> 7	80	100	100	85	90
GRO-α (pg/ml)	0 (0–389)	102 (0–474)*	> 70	60	80	75	67	70
ENA-78 (pg/ml)	186 (1–767)	1623 (197–3492)†	> 930	56	100	100	74	80
48 h after symptom onset								
CRP (mg/l)	80 (2·5–245)	215 (5–398)†	> 150	68	90	89	69	79
IL-8 (pg/ml)	0 (0–219)	49 (0–1686)†	> 7	72	90	90	73	80
GRO-α (pg/ml)	32 (0–698)	160 (0–2441)†	> 70	80	81	83	78	81
ENA-78 (pg/ml)	417 (23–2831)	1699 (103–7755)†	> 930	76	86	86	75	81
24 h after admission								
CRP (mg/l)	40 (2·5–167)	107 (11–343)†	> 150	35	93	85	56	65
IL-8 (pg/ml)	0 (0–16)	23 (0–369)†	> 7	71	94	92	75	81
GRO-α (pg/ml)	12·5 (0–389)	98 (0–1460)*	> 70	58	85	83	63	71
ENA-78 (pg/ml)	190 (8–812)	1812 (217–3942)†	> 930	75	100	100	79	87
48 h after admission								
CRP (mg/l)	73 (2·5–280)	228 (12–414)†	> 150	81	83	84	83	84
IL-8 (pg/ml)	0 (0–219)	49 (0–1686)†	> 7	70	91	90	73	80
GRO-α (pg/ml)	33 (0–698)	141 (0–3195)†	> 70	70	79	79	70	75
ENA-78 (pg/ml)	427 (7–10 808)	1699 (103–8089)†	> 930	78	91	91	78	84
Glasgow score	2 (0–5)	4 (0–7)†	> 2	74	86	87	86	79

Values are median (range). CRP, C-reactive protein; IL, interleukin; GRO, growth-related oncogene; ENA, epithelial neutrophil-activating protein; PPV, positive predictive value; NPV, negative predictive value. *$P < 0·05$, †$P < 0·01$ *versus* mild acute pancreatitis (Mann–Whitney *U* test) occurred in the 27 patients with a severe attack were circulatory failure in ten, metabolic failure in 20, renal failure in three, respiratory failure in 18, necrosis in six, pseudocyst formation in three and abscess in one. Eight patients died a median of 13 (range 5–14) days after symptom onset. The aetiology, APACHE II scores at recruitment and Glasgow scores 48 h after admission are shown in *Table 1*.

The plasma levels of CRP, IL-8, GRO-α and ENA-78 in mild and severe acute pancreatitis are shown in *Fig. 1* and *Table 2*. Plasma levels of CRP increased with time in both the mild and severe groups compared with those in normal healthy individuals, but were significantly greater in the severe pancreatitis group (*Fig. 1a*). CRP levels in patients with severe pancreatitis reached a peak at 3 days after onset of symptoms and then slowly decreased.

Plasma IL-8 levels were significantly raised in patients with severe acute pancreatitis from 24 h after symptom onset and remained high for 7 days after symptom onset (*Fig. 1b*). There was no difference in the plasma levels of IL-8 in patients with mild pancreatitis compared with those in healthy normal individuals (median 0 (range 0–157) pg/ml).

The plasma levels of both GRO-α (*Fig. 1c*) and ENA-78 (*Fig. 1d*) were raised 24 h after the onset of severe acute pancreatitis. The plasma levels of GRO-α in patients with a mild attack of acute pancreatitis were not significantly different from those in normal healthy individuals (median 0 (range 0–235) pg/ml). The levels of ENA-78 were significantly higher in patients with mild acute pancreatitis on days 2, 3 and 4 after the onset of symptoms than those in normal healthy individuals (median 148 (range 0–698) pg/ml). Median peak levels of CRP, IL-8, GRO-α and ENA-78 within the first 48 h of hospital admission were higher in patients who died than in those with severe disease who survived. None of these differences, however, reached statistical significance (data not shown).

The sensitivity, specificity, positive and negative predictive values and accuracy for the peak plasma levels of CRP, IL-8, GRO-α and ENA-78, 24 and 48 h following symptom onset, and 24 and 48 h following admission to hospital, are also shown in *Table 2*. Levels of all four inflammatory mediators were significantly increased in patients with severe compared with mild acute pancreatitis. Peak CRP within the first 24 h after admission had a low sensitivity (35 per cent) and accuracy (65 per cent) for the prediction of severe disease. In contrast, during the first 24 h after admission peak levels of IL-8 (accuracy 81 per cent), GRO-α (accuracy 71 per cent) and ENA-78 (accuracy 87 per cent) were better predictors of disease severity.

Discussion

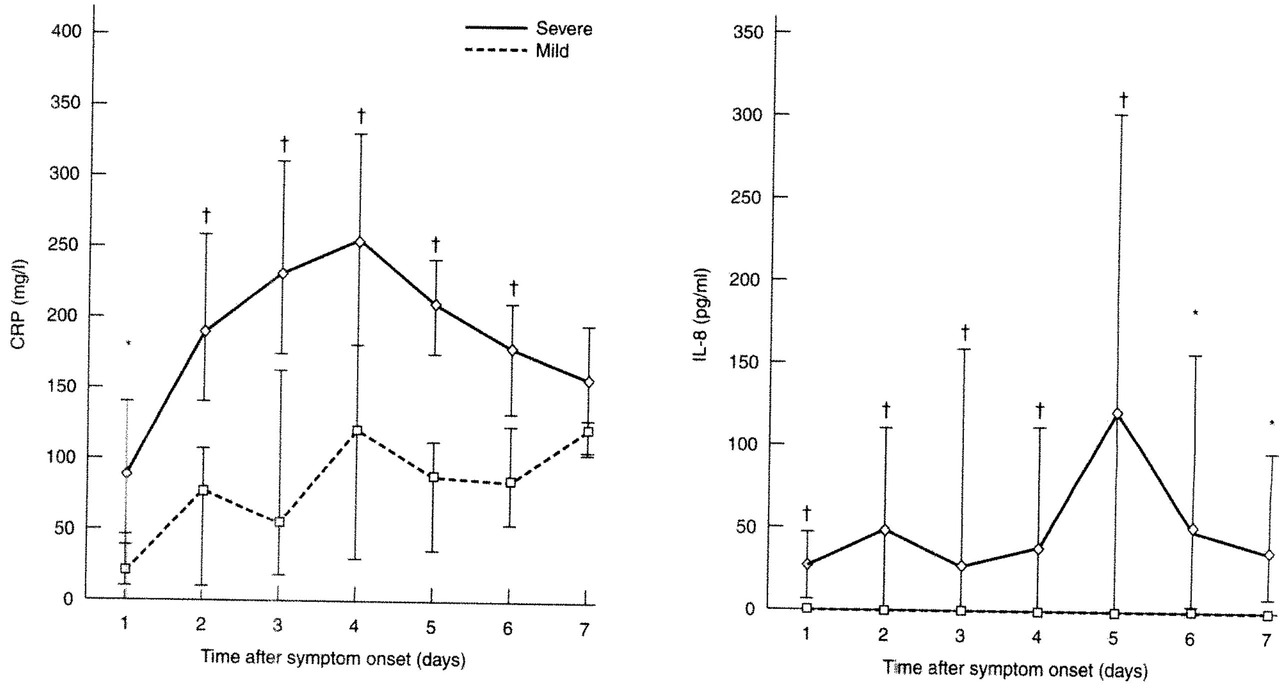
This study confirmed that plasma levels of IL-8 were raised following a severe attack of pancreatitis[12–16] and showed in addition that levels of GRO-α and ENA-78 were also increased. In patients with mild pancreatitis plasma levels of ENA-78 were raised but those of IL-8 and GRO-α were not.

Levels of all three chemokines were raised within the first 24 h after admission to hospital. Levels of IL-8 and GRO-α remained high for longer than those of ENA-78, which fell rapidly towards normal by the fourth day after symptom onset. Plasma levels of IL-8 have previously been shown in some[12,14], but not all[16], studies to peak at or soon after hospital admission, and to be a valuable early predictor of disease severity[13]. The results presented here suggest that ENA-78 was comparable to IL-8 as a predictor of severe disease during the first 24 h following admission to hospital.

The results of this study were expressed in terms of time from symptom onset, as this allowed a more accurate appreciation of temporal changes in cytokine levels and provided a standard for comparison between different series[25]. In this study the levels of GRO-α and ENA-78 as well as those of IL-8 were close to their peak 24 h following symptom onset, suggesting that they are all early mediators of the systemic inflammatory response in acute pancreatitis. In contrast, CRP plasma levels did not peak until 72–96 h following symptom onset. This is in keeping with previous studies[15,16,26] and helps to explain why CRP is accurate as a predictor of disease severity only at 48 h after hospital admission[25,27]. CRP measurement remains, however, an important standard biochemical test for the prediction of disease severity in acute pancreatitis because of its wide availability.

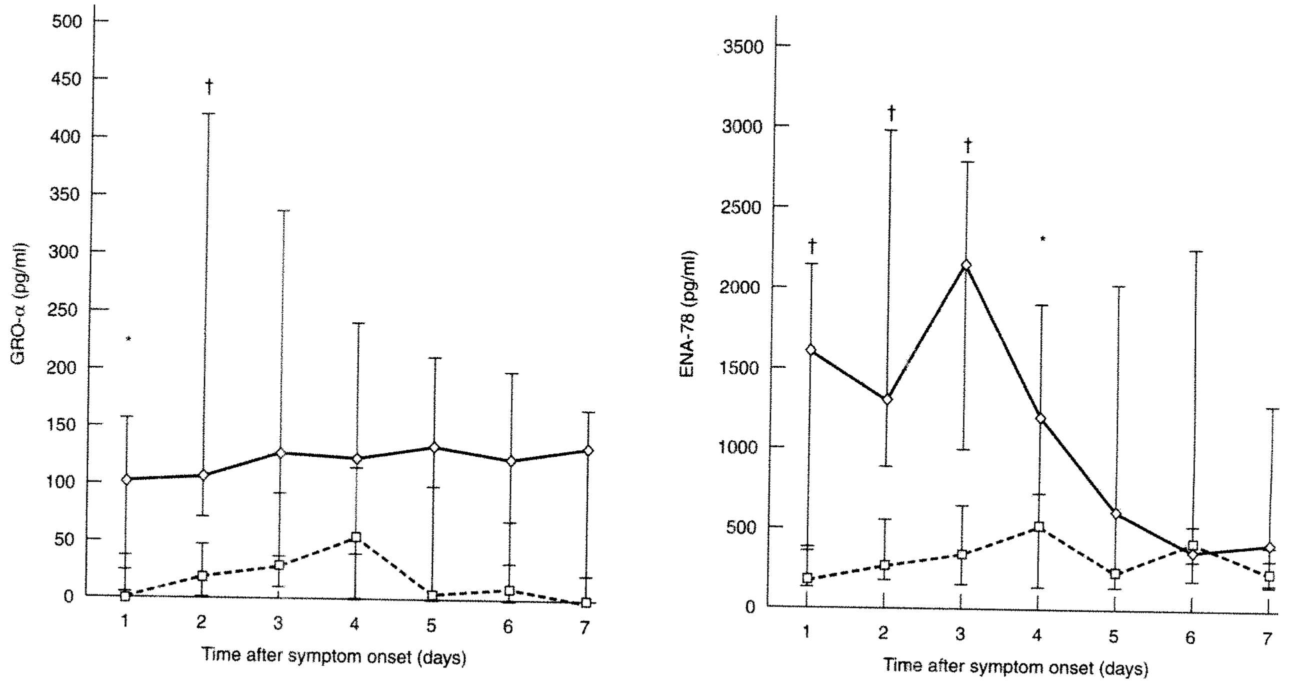
Levels of other inflammatory mediators including IL-10, the IL-1β receptor antagonist, procalcitonin and IL-6 are increased following an attack of acute pancreatitis and are each an accurate early predictor of the severity of an attack[5]. Raised systemic levels of these mediators reflect the magnitude of the inflammatory response, which in turn determines the severity of an attack. Whether the measurement of a single early inflammatory mediator could form the basis for a routine clinical test to detect at admission patients likely to suffer a severe attack is unclear, although IL-6 is the most studied candidate[28].

Neutrophils are thought to play a critical role in local and distant organ damage in acute pancreatitis since depletion of circulating neutrophils reduces both the severity of pancreatitis and the associated lung damage[29,30]. Neutrophil activation and migration into tissues is tightly regulated[4–7], and chemokines are now thought to play a key role. Pancreatic acinar cells may themselves be the source of



a C-reactive protein

b Interleukin 8



c Growth-related oncogene α

d Epithelial neutrophil-activating protein 78

Fig. 1 Median (interquartile range) plasma levels of **a** C-reactive protein (CRP), **b** interleukin (IL) 8, **c** growth-related oncogene (GRO) α and **d** epithelial neutrophil-activating protein (ENA) 78 on days 1 to 7 after symptom onset in severe and mild acute pancreatitis. *$P < 0.05$, †$P < 0.01$ *versus* mild acute pancreatitis (Mann–Whitney U test)

raised systemic chemokine levels but pancreas from patients with early acute pancreatitis is rarely available for study and evidence has to be sought from experimental studies.

IL-8 synthesis has been demonstrated in the pancreas of rabbits following induction of pancreatitis by bile salt infusion into the biliopancreatic duct[31]. In rats there is no direct homologue of IL-8, but cytokine-induced neutrophil chemoattractant (CINC), a homologue of human GRO-α, appears to be the major ELR-positive CXC chemokine. Synthesis of CINC in pancreatic acinar cells of rats follows induction of pancreatitis with either intraperitoneal injection of caerulein or bile salt infusion into the biliopancreatic duct; raised plasma levels of CINC follow within hours of induction in these models[32]. The raised plasma CXC chemokine levels seen in patients with severe acute pancreatitis are thus likely to be due to release of chemokines both from infiltrating leucocytes and from pancreatic acinar cells, as well as systemic leucocyte activation.

Raised plasma levels of all three chemokines, IL-8, GRO-α and ENA-78, predict severe acute pancreatitis which is characterized principally by distant organ injury, including that seen in the lungs. Further evidence that the chemokines are directly involved in organ damage in acute pancreatitis comes from experimental studies which show that treatment with the human monoclonal anti-IL-8 antibody (WS-4) decreases acute lung injury in a combined bile salt–duct ligation model of pancreatitis in rabbits[33], and systemic administration of an anti-CINC antibody reduces lung injury in caerulein-induced acute pancreatitis in rats[34]. Higher CXC chemokine levels may be associated with an increased magnitude of SIRS, MODS and early death. In the present study, levels of all three chemokines were higher in patients who died compared with survivors with severe pancreatitis, although these differences did not reach statistical significance. The number of patients who died in this study was small and so it is difficult to draw firm conclusions.

An understanding of the mechanisms that control leucocyte migration and activation may help in the development of measures to limit the inflammatory response and perhaps reduce organ damage in acute pancreatitis. The type of chemokine receptors expressed on circulating leucocytes, as well as the profile of chemokines present at a particular tissue site of inflammation or within the plasma, are likely to be important factors. How these interact and affect leucocyte activation and infiltration into organs in acute pancreatitis is, however, unclear; further work is required to characterize and correlate receptor expression on leucocytes with chemokine levels in plasma and tissues.

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Original article

Implications of peritoneal washing cytology in patients with potentially resectable pancreatic cancer

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Background: The aim of this study was to assess the implications of positive peritoneal washing cytology for management of patients with potentially resectable pancreatic cancer.

Methods: Cytological examination of peritoneal washings was performed in 134 patients who underwent surgical resection for pancreatic adenocarcinoma. The clinicopathological findings and the relationship between cytology results (including cytomorphology) and survival were investigated.

Results: One hundred and fourteen patients (85 per cent) had negative cytology results (group 1). Excluding one patient with atypical cells, positive cytology results were obtained in 19 patients (14 per cent): 16 patients without macroscopic peritoneal metastases (group 2) and three patients with minimal macroscopic peritoneal metastases (group 3). The patients in group 2 had significantly larger ($P < 0{\cdot}001$) and more advanced ($P = 0{\cdot}022$) tumours than those in group 1. However, there were no significant differences in postoperative cumulative survival rates between groups 1 and 2 ($P = 0{\cdot}347$). Two patients in group 2 are long-term survivors (40 and 58 months). In cytomorphological analyses, the presence of clusters with ragged edges and isolated carcinoma cells can be considered to indicate a high risk of peritoneal recurrence.

Conclusion: Positive cytology does not directly predict peritoneal carcinomatosis and, while associated with advanced disease, does not contraindicate radical surgery.

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Introduction

Pancreatic carcinoma is a disease with an extremely poor prognosis; fewer than 20 per cent of affected patients survive the first year, and only 3 per cent are alive 5 years after diagnosis[1]. Currently, surgical resection offers the only meaningful chance of cure for selected patients with local disease[1].

Peritoneal washing cytology has been widely used in the diagnosis and staging of malignant ovarian[2], endometrial[3] and gastric neoplasms[4]. In patients with pancreatic cancer, malignant cells have been identified in washes from 7 to 30 per cent of patients[5–8], but the prognostic significance of these findings has not been established. Several authors[7–11] have considered positive cytology to be an unfavourable prognostic factor that contraindicates further surgery. However, among published reports on peritoneal washing cytology for pancreatic cancer[5–12], there is little information about patients with positive cytology but no macroscopic metastases who have undergone resection. Up to the present at this institution, potentially resectable pancreatic carcinoma has been resected irrespective of the cytology results when macroscopic metastases are not found at laparotomy.

The purpose of this study was to determine whether positive cytology should contraindicate radical surgery in patients with potentially resectable pancreatic adenocarcinoma. Relationships between cytology results, including cytomorphology, and survival time and clinicopathological findings were investigated.

Patients and methods

Patients

Between January 1990 and December 1999, 344 patients were admitted to the National Cancer Center Hospital with a histologically confirmed diagnosis of pancreatic adenocarcinoma. Among them, 189 patients had unresectable disease by imaging criteria. The study population comprised a consecutive series of 134 patients who underwent surgical resection of pancreatic adenocarcinoma and who had cytology samples taken. There were 81 men and 53 women with a mean age of 62·7 (range 33–86) years, each of whom was followed up until death or to the present.

Surgery

Eighty-four tumours were located in the head of the pancreas, 46 in the body and/or tail and four diffusely involved the pancreas. The operations performed included pancreatoduodenectomy (63), pylorus-preserving pancreatoduodenectomy (21), distal pancreatectomy (39) and total pancreatectomy (11), with extended dissection of lymph nodes[13]. Histological evaluation revealed an R_0 resection in 86 patients and an R_1 resection (microscopic residual tumour) in 48 patients. In 93 patients intraoperative radiation therapy (30 Gy of electron beam radiation with an energy of 9 MeV) was administered to the retroperitoneal field[13]. Intraoperative radiation therapy was restricted to patients without contraindications who were under the age of 75 years. Of the 48 patients who underwent R_1 resection, 12 received postoperative adjuvant mitomycin C or 5-fluorouracil-based chemotherapy. Sixteen patients received similar treatment at the time of disease recurrence.

Peritoneal washing cytology

Specimens for cytological examination were obtained before manipulation of the tumour at laparotomy. Normal saline solution (100 ml) was instilled into the pelvis with a bulb syringe. The specimens (20 ml) were aspirated from the cul-de-sac (pouch of Douglas) after abdominal agitation into a sterile tube containing sodium ethylenediamine tetra-acetic acid. They were first mixed thoroughly and then divided into six equal chambers for cytocentrifuge preparation. The cells were centrifuged in a Sakura Autosmear CF-120 cytocentrifuge (Sakura Finetechnical, Tokyo, Japan) at 1500 r.p.m. for 70 s and transferred to six glass microscope slides, two for Papanicolaou staining, one for Giemsa staining, one for Alcian blue staining and the remaining two for immunocytochemical staining. The diagnosis was made using a standard cytological technique (Papanicolaou, Giemsa and Alcian blue staining) on the basis of the cytomorphological features. Immunocytochemistry was performed to help establish the diagnosis with antibodies against carcinoembryonic antigen (CEA, dilution 1 : 5000; Mochida Pharmaceutical, Tokyo, Japan)[12,14] and MOC-31 (dilution 1 : 100; Dako, Glostrup, Denmark)[15] or Ber-EP4 (dilution 1 : 50; Dako)[16]. Positive and negative controls were performed on each staining run. The findings of CEA, MOC-31 and Ber-EP4 reactivity were all 100 per cent sensitive, and 93, 91 and 100 per cent specific respectively.

Cytomorphological examination

Positive cytology showed a variety of morphological features, raising the possibility that these may be related to the clinical course of the disease. The number of clusters, their size, the presence of isolated carcinoma cells, the predominant contour shape of the clusters (ragged or well defined edges) and the presence of cytoplasmic vacuoles were therefore recorded. The number of clusters was classified as small (less than five clusters per slide), medium (between five and 20 clusters per slide) or large (more than 20 clusters per slide). Size was defined as small (largest cluster consisting of fewer than 50 cells; *Fig. 1a*), medium (largest cluster consisting of 50–500 cells; *Fig. 1b*) and large (largest cluster consisting of more than 500 cells; *Fig. 1c*). Typical peritoneal cytology showing clusters with ragged edges is illustrated in *Fig. 2*.

Histopathological examination

The resected specimens were fixed in 10 per cent buffered formalin and cut serially into slices 3–5 mm thick. Histopathological examination was carried out to determine the size and location of the tumour, histological type and differentiation, lymphatic invasion, venous invasion, intrapancreatic nerve invasion, invasion of the anterior pancreatic capsule (serosal invasion), retroperitoneal tissue, portal venous system and arterial system, and staging. The histological type and differentiation were determined according to the Classification of Pancreatic Carcinoma of the Japan Pancreas Society[17]. The Union Internacional Contra la Cancrum tumour node metastasis staging system was applied for the pathological staging of each carcinoma[18].

Statistical analysis

Data were expressed as mean(s.d.), and statistical analysis was performed using Student's t test or χ^2 test as appropriate. Survival analysis was performed by the Kaplan–Meier method, and survival curves were compared with the log rank test.

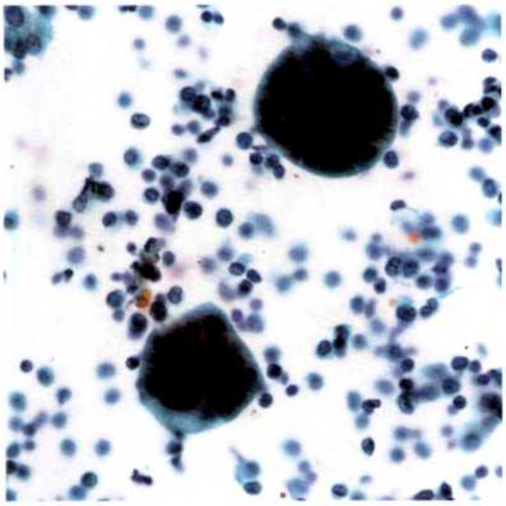
Results

Cytological examination of peritoneal washings showed malignant cells in 19 patients (14 per cent). One patient was diagnosed as having atypical cells, since CEA- and MOC-31-positive cells were present but their nuclei were small with only mild atypia. Three patients with positive cytology had a few macroscopic peritoneal metastases. All three patients underwent resection of the macroscopic metastatic nodules and distal pancreatectomy. Thus, excluding the patient with atypical cells, the patients were divided into three groups: those with negative cytology (group 1; 114 patients), patients with positive cytology but no macroscopic peritoneal metastases (group 2; 16 patients) and those with positive cytology and macroscopic peritoneal metastases (group 3, three patients).

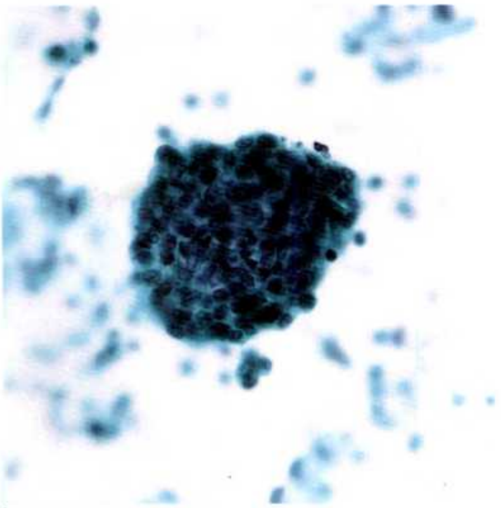
In group 1 there were 103 tubular adenocarcinomas (33 well differentiated, 55 moderately differentiated and 15 poorly differentiated), five papillary adenocarcinomas, two mucinous carcinomas, two invasive carcinomas derived from intraductal papillary adenocarcinomas[19], one adenosquamous carcinoma and one mucinous cystadenocarcinoma. Carcinomas in group 2 comprised 13 tubular adenocarcinomas (seven well differentiated, five moderately differentiated and one poorly differentiated), two invasive carcinomas derived from intraductal papillary adenocarcinomas and one papillary adenocarcinoma. All three tumours in group 3 were moderately differentiated tubular adenocarcinomas. Tumour size (greatest dimension) in group 2 (6·0(2·8) cm) was significantly greater than that in group 1 (4·1(1·9) cm) ($P < 0\cdot001$).

Patients in group 2 had a significantly higher incidence of invasion of the anterior pancreatic capsule (eight of 16 *versus* 11 of 114; $P < 0\cdot001$) and retroperitoneal tissue (ten of 16 *versus* 38 of 114; $P = 0\cdot024$) than those in group 1. Eight of the 16 patients in group 2 were diagnosed as positive for invasion of the anterior pancreatic capsule. Conversely, among 19 patients who had histologically proven tumour invasion of the anterior pancreatic capsule, eight had positive cytology. Fourteen of 16 patients in group 2 had stage IV disease and the remaining two patients had stage II cancer. Statistical analysis showed advanced tumours to be more common in group 2 than group 1 ($P = 0\cdot022$).

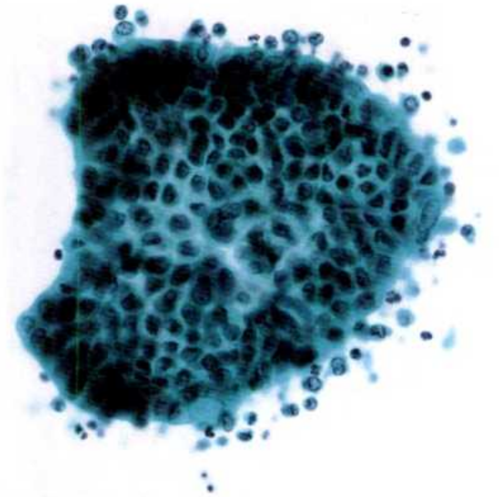
The 1-, 3- and 5-year cumulative survival rates of all patients after surgery (excluding four perioperative deaths) were 65·5, 32·2 and 23·7 per cent respectively. *Fig. 3* illustrates the survival of patients in group 2 compared with those in group 1. There was no statistically significant difference in the postoperative cumulative survival rate between the two groups ($P = 0\cdot347$). Two patients in group 2 are currently long-term survivors (40 and 58 months).



a Small clusters



b Medium-sized clusters



c Large cluster

Fig. 1 Examples of cell clusters of each size in peritoneal washing cytology samples. **a** Two small clusters, **b** medium-sized cluster and **c** large cluster of pancreatic adenocarcinoma cells (Papanicolaou stain, original magnification × 400)

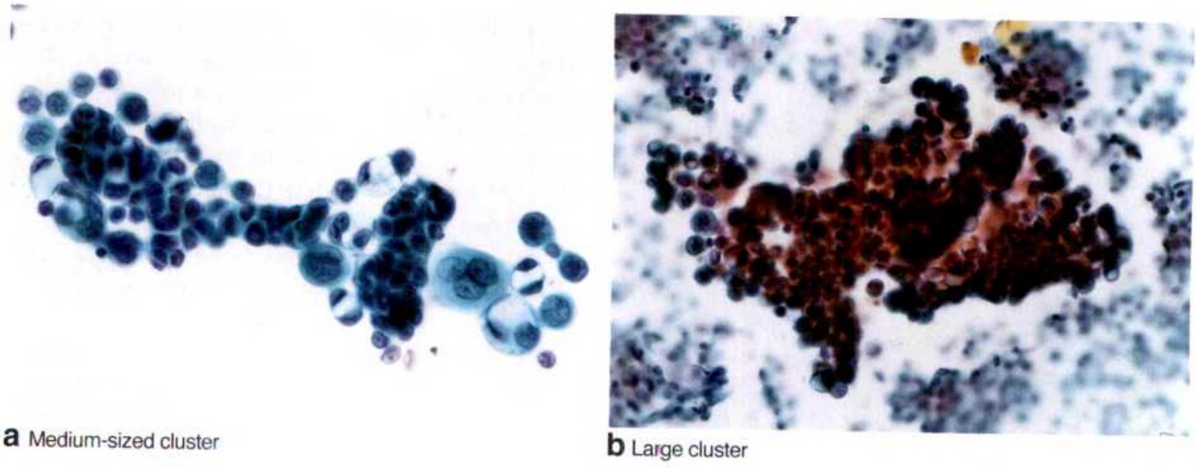
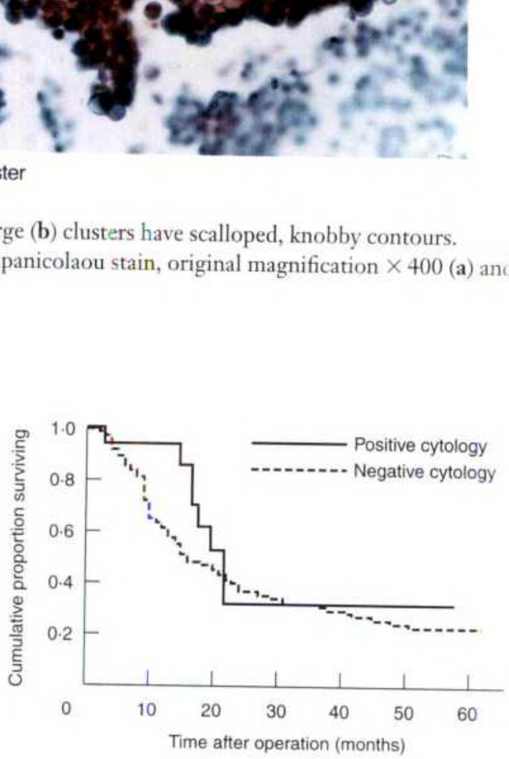

a Medium-sized cluster **b** Large cluster

Fig. 2 Examples of cell clusters with ragged edges. The medium-sized (**a**) and large (**b**) clusters have scalloped, knobby contours. Isolated carcinoma cells are becoming detached from the clusters at the edges (Papanicolaou stain, original magnification × 400 (**a**) and × 200 (**b**))

Cytological preparations of pancreatic carcinomas from different patients exhibited various cytomorphological patterns and features, but the findings reflected the morphology of the primary pancreatic cancer in each case. Tumour cells with intracytoplasmic vacuolation seen on peritoneal washing cytology were also encountered in mucin-producing adenocarcinomas. In well differentiated carcinomas, although the size of the cell clusters varied, tumour cells tended to form three-dimensional, tightly packed, cell clusters. In contrast, poorly differentiated carcinoma, or carcinomas invading the peripancreatic tissue in loose groupings and/or as single cells, visible at the advancing edge on histological sections, revealed clusters with ragged edges (scalloped, knobby contours) and isolated carcinoma cells on peritoneal washing cytology.

All patients in group 2 showed either a small or medium number of clusters, although the size of the cell clusters varied, whereas all three patients in group 3 had a medium or large number of clusters. Seven of the 19 patients in groups 2 and 3 had clusters with ragged edges, concomitantly with isolated carcinoma cells. Clusters with ragged edges and isolated carcinoma cells were identified in four of five patients who suffered disseminated peritoneal recurrence after surgery in group 2. Two patients in group 3 who exhibited similar findings died at 3 and 7 months.

Review of the cytomorphological findings in peritoneal washings from four patients who had macroscopic peritoneal metastases at the time of laparoscopy or laparotomy and did not undergo pancreatectomy revealed a large number of clusters, cell clusters with ragged edges and isolated carcinoma cells in all cases.



No. at risk

Positive cytology	15	13	6	2	2	1	0
Negative cytology	111	77	45	30	24	18	15

Fig. 3 Correlation of the postoperative cumulative survival rate with cytological findings in the peritoneal washings of patients without macroscopic peritoneal metastases (excluding perioperative deaths)

Discussion

It has been reported that positive peritoneal washing cytology in patients with pancreatic cancer correlates with advanced stage, early metastasis and short survival[5–12]. The results of this study confirm that the presence of malignant cells in peritoneal washes is associated with advanced disease and a larger tumour size. Several authors[7–12] have evaluated the significance of positive cytology in the absence of gross metastases in patients with pancreatic cancer. In the report by Makary *et al.*[9], the median survival time of 15 patients with positive cytology without macroscopic metastases was 8·6 months (only two patients underwent pancreatectomy), whereas that of 30 patients with negative cytology was 13·5 months. Recently, Z'graggen *et al.*[20] reported a high prevalence of circulating tumour cells in patients with cytological evidence of free cancer cells in the peritoneal cavity. Most studies[7–11] concluded that positive cytology contraindicates further treatment, or at least surgery, for these patients. However, this conclusion may be premature since, among the entire series of published reports[5–12], only 17 patients found to have positive cytology underwent potentially curative resection. In the present study, there was no significant difference in postoperative cumulative survival rates between patients with positive and negative cytology. Nakao *et al.*[12] reported similar findings in 13 patients with positive cytology and 37 patients with negative cytology who had resectable cancer but no macroscopic peritoneal metastases. Furthermore, in the current study the majority of patients with positive cytology did not develop peritoneal carcinomatosis in the early postoperative period.

There are reports of long-term survival (more than 3 years) after surgery[10,12], even in the presence of positive cytology, as was the case with two of the present patients. Most of the patients in group 2 had a large but well differentiated and potentially resectable tumour, which may indicate an exceptional, and relatively favourable, form of pancreatic cancer. The results do not point to cytological findings as an independent risk factor for survival, and a positive result did not appear to signify non-resectability. It is therefore the authors' view that when a pancreatic lesion is localized or macroscopically resectable without evidence of distant metastasis, it should be resected, even if peritoneal washing cytology is positive, as curative resection may offer the best chance for long-term survival in these patients.

It has been reported that in gastric and colorectal cancer, even at stage I when direct tumour access can be excluded completely, approximately 22 and 30 per cent of patients respectively have circulating tumour cells within the peritoneal cavity[21]. This observation is in line with the current findings for pancreatic cancer. Although the majority of intraperitoneal free cancer cells are presumed to be released from areas of pancreatic serosal invasion, some may reach the peritoneum via lymphatic channels as occurs in gastric cancer[22].

Not all cancer cells present in the peritoneal cavity generate peritoneal metastatic lesions[23]. Many factors, such as the number and viability of the cells, influence the potential for forming metastases. The present study suggests that positive cytology and peritoneal carcinomatosis are not always concurrent in patients with pancreatic cancer.

The relationship between the cytomorphological features of patients with positive cytology and the histology of resected specimens was investigated; the number of clusters, the presence of isolated carcinoma cells and the contour shape of clusters (ragged or well defined edges) were important. When the number of intraperitoneal free cancer cells is large, the chance of successful implantation might be expected to increase. Although the cytomorphological appearance of clusters might be partly an artificial phenomenon due to centrifugation, it seems likely that peritoneal washing cytology findings faithfully mirror the morphology of the primary pancreatic cancer. The ragged edges of clusters indicate a tendency for cells to separate and a close association with isolated carcinoma cells[24]. These cytological features probably imply poor cohesion among the cells, linked to dysfunction of cell–cell adhesion, for example alteration of E-cadherin[25,26] and associated cytoplasmic proteins, α-, β- and γ-catenins[27]. Such dysfunction may not only correlate with poor differentiation but also increased invasiveness[25–29].

In conclusion, positive peritoneal cytology is associated with advanced disease in patients with pancreatic cancer but does not always contraindicate radical surgery. Cytological findings of clusters of cells with ragged edges and isolated carcinoma cells may indicate a high risk for intra-abdominal recurrence.

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Original article

Electrolytic ablation as an adjunct to liver resection: experimental studies of predictability and safety

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Background Combined liver resection and local ablation may offer the only chance of cure to patients with liver metastases who are presently deemed unresectable because of a single awkwardly placed metastasis. By definition, such a metastasis is often close to a major vein. An ablative technique is needed that is both predictable and safe in such a circumstance.

Methods Electrolytic liver lesions were created in 21 pigs using platinum electrodes, connected to a direct current generator. Both electrolytic 'dose' and electrode separation were varied to produce different sized lesions. The 'dose' was correlated with the volume of necrosis and any vascular damage was determined histologically.

Results There was a significant ($P < 0\cdot001$) correlation between the electrolytic 'dose' and the volume of liver necrosis. For a given 'dose' the volume of necrosis was less when the electrodes were together, rather than separated. Liver enzymes were only transiently deranged. There were no significant vascular injuries.

Conclusion Predictable and reproducible necrosis is produced by electrolysis in the pig liver. The treatment appears to cause little or no damage to immediately adjacent liver or major vascular structures and, when combined with resection, may offer the chance of a cure to many patients who are currently unresectable.

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Introduction

Surgical resection remains the only potentially curative treatment for patients with colorectal liver metastases. Without treatment the prognosis is poor[1], with a median survival of 6 months[2,3]. Only approximately 20 per cent of such patients are suitable for liver resection for a variety of reasons[4]. Patients are often deemed unresectable because of one or two metastases that are awkwardly placed close to a major vein. If such a lesion could be treated with local ablation, the patient may then become resectable and potentially curable.

Several methods of local ablation have been reported, including radiofrequency ablation[5,6], cryotherapy[7], interstitial laser therapy[8], microwave hyperthermia[9] and direct injection of toxic agents such as alcohol[10], acetic acid[11] and hot saline[12]. The majority of these methods use extremes of temperature to cause tumour necrosis. However, all thermal methods of ablation are limited in their use close to major intrahepatic veins because of a combination of the 'heat-sink' effect[13,14] and direct damage to the vein. In the 'heat-sink' phenomenon, the thermal insult is reduced close to major vascular structures owing to the effect of local blood flow. As a result, if a tumour is adjacent to a major vein, a cuff of viable tumour may be left untreated around the vessel[15]. Conversely, the thermal insult may actually cause a direct 'burn' to the vessel. Indeed with cryotherapy, cracks may form in the ice-ball during freezing. As thawing begins, any involved hepatic veins may then bleed, causing potentially fatal haemorrhage[16,17]. Cryotherapy is also associated with the 'cryoshock' phenomenon in which patients develop a systemic inflammatory response to the products of cell necrosis. Patients have died as a direct result of this phenomenon[18,19]. Additionally it has been shown that radiofrequency ablation may achieve incomplete tumour necrosis within the treated area[20,21].

Clearly, if a locally ablative treatment is to be used as an adjunct to surgical resection, it is crucial that it is: (1) predictable in effect; (2) safe and effective close to major veins; and (3) achieves complete necrosis of the treated tumour.

Electrolytic ablation was first described by Nordenstrom[22,23] for the treatment of lung tumours.

However it was not until recently that this method was applied to the local ablation of liver tumours[24]. Rather than relying on a rapid 'burn', electrolysis causes cell death by a more subtle, yet equally effective, chemical action. Platinum electrodes are inserted into the tumour and a small direct electrical current is passed between them, thereby polarizing the electrodes. As a result, negatively charged ions are attracted to the anode and positively charged ions to the cathode, resulting in a change in intracellular pH of the tissues surrounding the electrode tips. Additionally, cytotoxic gases (such as chlorine) are liberated into the tumours and necrosis results from a combination of these two effects; importantly, there is no heating effect[25].

Previous studies[24,26] have shown that electrolysis is a safe, predictable, reproducible and controlled method for producing spherical areas of hepatic necrosis in the rat. Colorectal liver metastases were also ablated using this technique in a rat model[27]. Importantly, more recently it has been shown that electrolytic treatment caused little or no damage to hepatic veins when the electrode catheters were placed either directly adjacent to or within the lumen of the vein[28]. In this study of six pigs, only one animal developed thrombosis of the hepatic vein and there was no evidence of endothelial damage in any of the animals, even though the necrotic lesion directly abutted the vein wall.

Because of this lack of vascular damage, electrolysis would appear to be ideally suited to the treatment of perivascular liver metastases. However, before treating patients it is essential that the extent of the necrosis can be predicted accurately by the electrolytic 'dose' given using data generated from a large animal model with a liver of similar physical size to that of the human. This study examined the effects of varying both the electrolytic 'dose' and electrode separation on the volume of the resultant electrolytic lesion(s) in normal pig liver and any associated vascular damage.

Materials and methods

Twenty-one female Domestic White pigs (24–33 kg) were used. All animals were anaesthetized in the same way; the pigs were sedated with a deep intramuscular injection of ketamine 20 mg/kg and xylazine 1·5 mg/kg. Anaesthesia was maintained using a laryngeal mask airway with 1·5 per cent halothane in 100 per cent oxygen. The oxygen saturation, pulse rate and end-tidal carbon dioxide were monitored throughout the procedure. Each animal underwent electrolysis with electrodes 'together' (12 mm separation) and 'separated' (200 mm separation).

During the early part of the study, intraoperative ultrasonography was used in an attempt to monitor the evolution of the electrolytic lesions. However, this was abandoned as the production of gaseous electrode products produced a 'snowstorm' effect, making images uninterpretable.

Electrodes together (12 mm)

The liver was exposed through an upper midline incision. A single 6-Fr (2 mm) electrode catheter (Johnson & Johnson Medical, North Ryde, New South Wales, Australia) (*Fig. 1*) was inserted centrally into the dome of the left lobe to a depth of 30 mm, such that the electrodes were in close proximity to the left hepatic vein. The tip electrode (anode) and the middle of the three proximal electrodes (cathode) were connected to the direct current (DC) generator (Bioengineering, Transducers and Signal Processing Research Group, University of Leicester, Leicester, UK), giving an electrode separation of 12 mm. The unused electrodes were electrically isolated. The electrolytic 'dose' (25–300 coulombs (C), delivered at 30–80 mA) was then given. Depending on the 'dose' delivered, treatment time varied from 8 to 100 min. At the end of treatment, the electrode catheter was removed. The resulting 'composite' lesion consisted of two distinct but overlapping anode and cathode lesions.

Electrodes separated (200 mm separation)

Using the same animal, two separate electrode catheters were inserted adjacent to major veins; the anode was inserted centrally in the right lobe and the cathode was inserted centrally in the left lobe, giving an electrode separation of 200 mm. The tip electrodes of each catheter were connected to the DC generator and the same electrolytic 'dose' was delivered. After treatment the electrodes were removed.

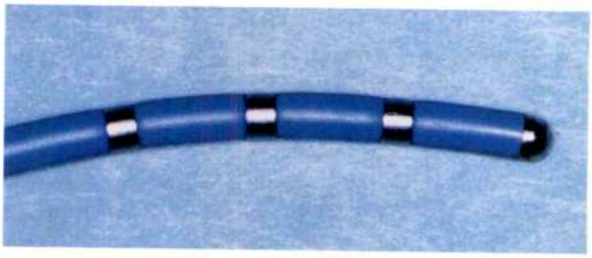

Fig. 1 A 6-Fr (2 mm) platinum electrode catheter. The tip electrodes of two catheters were used when the electrodes were separated. The tip and middle electrodes of a single catheter were used when the electrodes were placed together

Analysis

All animals were killed 72 h after treatment by lethal injection.

Blood samples were obtained before operation, 24 h after treatment and at the time of death. Serum measurements of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyltranspeptidase (γGT) and bilirubin were made on each sample (Technicon Axon analyser; Bayer Health Care, Pymble, New South Wales, Australia). At autopsy, the liver was removed, and the electrolytic lesions were excised *en bloc* and fixed in 10 per cent formalin.

After 2 weeks' fixation, each electrolytic lesion was cut into 1-mm sections in its entirety (*Fig. 2*). Sections were then measured blindly by two separate investigators. For each lesion, the three maximum diameters at right angles to each other were measured and recorded. The mean of the observer's measurements for the three diameters was calculated. The volume of each lesion was then calculated.

Each electrolytic lesion was processed, embedded in paraffin, sectioned and stained using haematoxylin and eosin. Coded sections were examined by a hepatopathologist to determine both the extent of necrosis and any associated vascular or biliary damage.

Results were analysed statistically using the paired *t* test and regression analysis.

Results

No animal died as a result of electrolysis or had to be killed prematurely. Treatment was uneventful and all animals made a rapid and full postoperative recovery.

Electrodes together (12 mm separation)

The volume of necrosis increased with the electrolytic 'dose'. There was a significant correlation between the volume of the electrolytic lesion and the 'dose' delivered ($r^2 = 0{\cdot}64$, $P < 0{\cdot}001$) (*Fig. 3*). The relationship was linear, although with doses greater than 75 C, the variability in the volume of the lesion increased. The rate at which the treatment was given (milliamperes) had no significant effect on this relationship. At the maximal 'dose' of 300 C, lesions of 2·05 cm in diameter were created.

Electrodes separated (200 mm separation)

Anode lesions

There was a significant correlation between the volume of the electrolytic lesion and the 'dose' delivered ($r^2 = 0{\cdot}91$, $P < 0{\cdot}001$) (*Fig. 4*). The rate at which the treatment was given again had no significant effect on this relationship.

Cathode lesions

As with the anode lesions there was a significant ($r^2 = 0{\cdot}73$, $P < 0{\cdot}001$) correlation between the volume of the electrolytic lesion and the 'dose' delivered (*Fig. 4*). The rate at which the treatment was given had no significant effect on this relationship. At the maximal 'dose' of 300 C lesions of 2·75 cm were created.

Comparison of electrode arrangements

Fig. 5 shows the mean volume of necrosis per coulomb delivered with the electrodes together or separated. For a

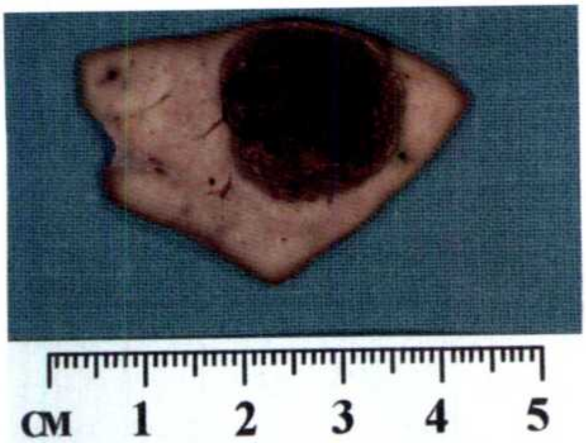

Fig. 2 Section (1 mm) of an electrolytic lesion created at the anode using 200 C

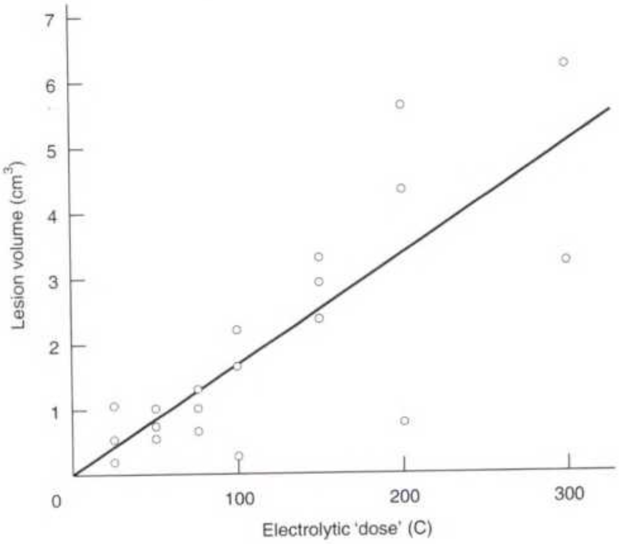

Fig. 3 Correlation between the volume of the composite lesion and the electrolytic 'dose' delivered ($r^2 = 0{\cdot}636$, $P < 0{\cdot}001$)

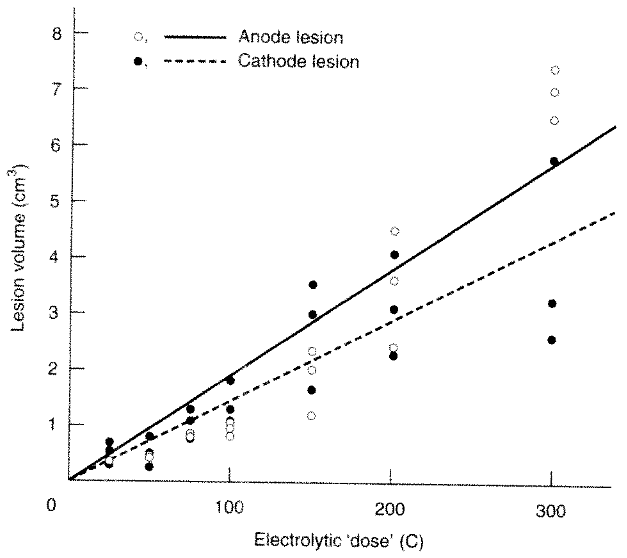

Fig. 4 Correlation between the volume of necrosis and the electrolytic 'dose' delivered for both anode (r^2 = 0·91, $P < 0·001$) and cathode (r^2 = 0·73, $P < 0·001$)

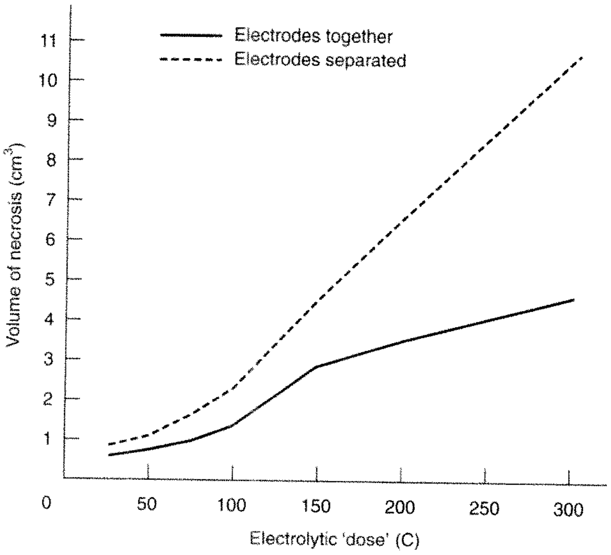

Fig. 5 Mean volume of necrosis with the electrodes together or separated

given 'dose', the volume of necrosis produced was greater when the electrodes were separated; this disparity increased with higher doses.

Liver enzymes

Levels of liver enzymes (AST, ALT and γGT) were significantly raised 1 day after treatment ($P < 0·001$ for AST) (*Fig. 6*). The initial increase in AST was significantly correlated with both the volume of the anode lesions (r^2 = 0·54, $P < 0·001$) and the total volume of liver necrosis (r^2 = 0·38, $P < 0·01$). Similarly, the initial increase in ALT was significantly correlated with both the volume of the anode lesions (r^2 = 0·45, $P < 0·001$) and the total volume of liver necrosis (r^2 = 0·26, $P < 0·05$). There was no correlation between either the cathode or composite (electrodes together) lesions and the increase in liver enzyme levels. At the time of death the liver enzymes had returned to normal apart from the AST level which remained significantly raised ($P < 0·05$).

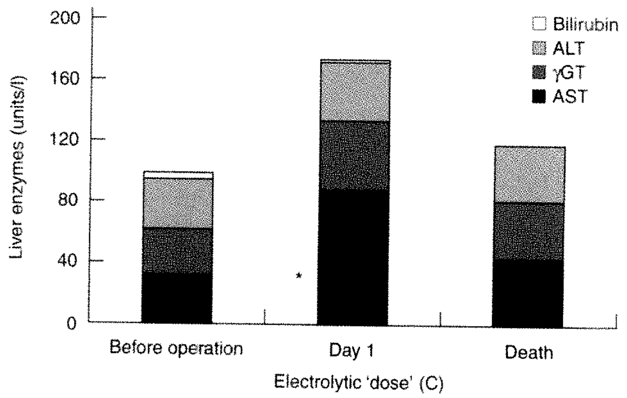

Fig. 6 Change in liver enzyme levels after electrolysis. ALT, alanine aminotransferase; γGT, γ-glutamyltranspeptidase; AST, aspartate aminotransferase. *$P < 0·001$ (AST; before operation to day 1; paired t test)

Histopathology

Histological examination confirmed the macroscopic observation that the size of the electrolytic lesion increased with the dose delivered. Each lesion consisted of a central zone of coagulative necrosis around the site of the electrode tip. No viable tissue was seen within the electrolytic lesions. The necrotic tissue was sharply demarcated from the surrounding liver tissue, which was histologically normal (*Fig. 7*).

Small blood vessels (less than 1 mm) were thrombosed in a narrow rim (0·5–1·0 mm) of normal liver surrounding each lesion. Only one major vein was thrombosed. In all other specimens, veins directly adjacent to electrolytic lesions were undamaged (*Fig. 8*) and there were no major bile duct injuries.

Discussion

Previous studies in both small and large animal models[26,29] have demonstrated that electrolytic lesions in the liver heal

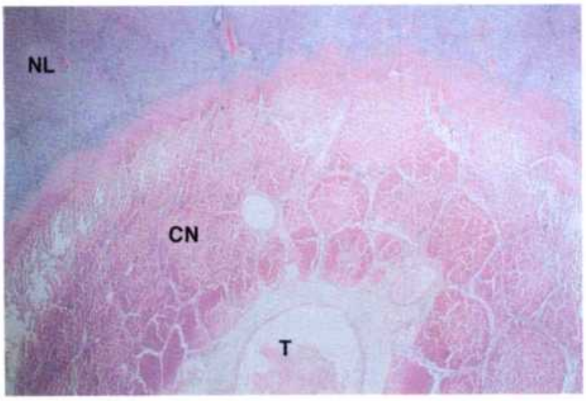

Fig. 7 Histological appearances. There is a circular area of tissue destruction at the site of the electrode tip (T) surrounded by a circular zone of coagulative necrosis (CN), with sharply demarcated normal liver at the periphery (NL). (Haematoxylin and eosin stain, original magnification × 4)

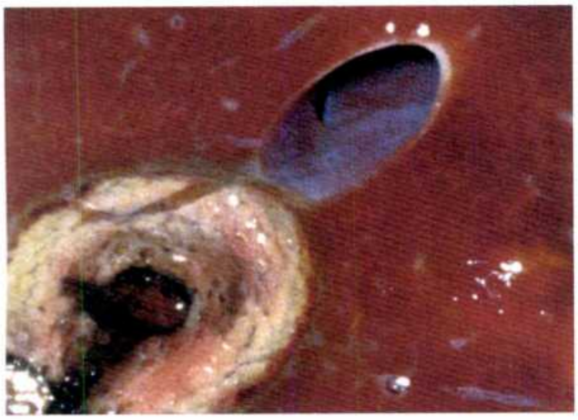

Fig. 8 Undamaged, patent hepatic vein, directly adjacent to an electrolytic (cathode) lesion

with time without complications. Six months after treatment little necrotic tissue remained at the site of the original lesion, which was replaced by a small fibrous scar. It was also shown that colorectal liver metastases as well as normal liver tissue could be destroyed completely by this technique[27].

If the treatment is to be consistently associated with minimal morbidity and mortality rates, however, it must be demonstrated that the effect of treatment is both predictable and reproducible. To date the dose–response data in the literature regarding electrolysis are limited and variable. However, one study has reported experimental dose–response data in rabbit liver[30]; liver necrosis was produced at a rate of 2·4 cm^3 per 100 C compared with a rate of 4·1 cm^3 for lung necrosis in the same study. In a recent study[24] it was demonstrated that electrolytic necrosis in rat liver was produced at a rate of approximately 2·0 cm^3 per 100 C when the electrodes were placed together and 3·4 cm^3 per 100 C when the electrodes were separated. In anecdotal reports of patients with large hepatocellular carcinomas treated with electrolysis, Chinese workers have reported completely different rates of destruction with two orders of magnitude difference in response (between 485 and 837 cm^3 per 100 C)[31,32]. Before treating patients, however, it is essential that reliable dose–response data are generated using a large animal model with a liver of similar physical size to that of the human.

The present results show that the volume of necrosis produced by a given electrolytic 'dose' is both predictable and reproducible in pig liver. However, the effect is greater, and the final lesion larger, when the electrodes are separated by a distance of 200 mm rather than with minimal separation. This finding is consistent with other experimental studies[33,34]. It is likely that this discrepancy results from a combination of factors. One such factor may be that when the electrodes are very close the cytotoxic gases generated from each electrode mix, effectively cancelling out their respective effects[34].

In this study, a maximum 'dose' of 300 C was used. With the electrodes separated, this produced two lesions with a combined volume of nearly 11 cm^3, or individual diameters of 2·2 cm. In clinical practice metastases are often larger than this. However, there is no reason to suggest that electrolytic 'doses' far in excess of 300 C could not be used safely. Indeed, as a part of a pilot study in patients, 1500 C has been given to a patient with a hepatoma, without adverse effects.

Histological examination of all the specimens in this study demonstrated three important beneficial effects of electrolytic ablation that may be of relevance when treating perivascular metastases. First, no viable tissue remained in the area treated by electrolysis in any of the lesions that were created. There was 100 per cent necrosis of the cells that were contained within the macroscopically apparent electrolytic lesion. Additionally, there was a very sharp 'transition zone' between the area of total necrosis and normal surrounding liver. This finding is in contrast to the results of radiofrequency ablation where recurrence has been reported within the treated area, presumably resulting from incomplete ablation at the time of treatment[20,21].

Second, although there was an hepatic vein occlusion in one specimen, there was no histological evidence of major vascular or biliary damage in any of the remaining specimens, despite the close proximity of many lesions to hepatic veins. If local ablation is intended for use in this situation, these results support the view that electrolysis may be more efficacious than treatments such as cryotherapy and radiofrequency ablation.

Third, although there was virtually no damage to large veins as a result of electrolysis, each lesion was surrounded by a narrow rim of normal liver in which the small sinusoids were thrombosed. It is proposed that these small, occluded vessels may form a 'cocoon' around the necrotic lesion. This vascular isolation may in turn prevent the rapid efflux of necrotic debris into the systemic circulation and hence avoid the systemic inflammatory reaction that is known to occur with cryotherapy[18,19]. This theory requires further evaluation but is supported by the lack of a systemic response in experimental studies to date[26,28,29].

Levels of liver enzymes were significantly raised after treatment and the magnitude of the increase correlated with the total volume of liver necrosis. However, while the increase in liver enzymes was statistically significant, there were no clinical sequelae and no animal developed hepatic failure or became jaundiced after treatment. Indeed, 72 h after treatment the enzyme levels had either returned to or were returning to normal. The local trauma associated with electrolysis appears to be minimal and transient.

Platinum electrodes were used in this study because it has been shown that they are durable and not readily eroded by the electrolytic process[21]. Furthermore, it has been suggested that the use of platinum electrodes may increase the toxicity of the local environment by the production of cytotoxic platinum salts[35–38]. This remains theoretical and unproven, and requires further evaluation. The potential toxicity of platinum salts and other products of electrolysis entering the systemic circulation has been investigated[28]. No systemic toxic effects were observed either during or after treatment.

This study has confirmed that electrolysis fulfils many of the criteria for an ablative technique which may be used as an adjunct to surgery to make the 'unresectable' patient 'resectable' by treating one or two awkwardly placed metastases. In this study electrolytic ablation produced predictable areas of necrosis with no significant complications, close to major vascular structures. Additional theoretical advantages, such as the lack of a systemic inflammatory reaction and the local production of cytotoxic platinum salts, require further evaluation.

Presently, electrolytic ablation is performed at laparotomy. However, there is no reason why the treatment could not be performed percutaneously in the future, as delivery systems are currently available which would accept the 6-Fr catheters used in this study. Based on the encouraging results of this study, preliminary clinical trials have started and several patients who would otherwise have been deemed inoperable have been treated successfully.

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Impact of socioeconomic deprivation on outcome after surgery for colorectal cancer

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Background: Previous studies have demonstrated that socioeconomic deprivation is associated with poorer survival in patients with colorectal cancer. These differences have been attributed to more advanced disease at presentation.

Methods: A total of 2269 patients undergoing resection for colorectal cancer in hospitals in central Scotland between 1991 and 1994 were studied. Socioeconomic status was defined using the Carstairs deprivation index. The impact of deprivation on case mix, treatment and outcome was analysed.

Results: There were no significant differences in mode of presentation, extent of disease at presentation, type of resection and postoperative mortality rate among the socioeconomic groups. Following curative resection, the overall survival rate at 5 years was 47·0 per cent in deprived patients, compared with 55·4 per cent in affluent patients ($P = 0·05$); the cancer-specific survival rate was 62·6 per cent in the deprived and 68·1 per cent in the affluent ($P = 0·05$). Compared with the affluent, the adjusted hazard ratios for the deprived were 1·36 (95 per cent confidence interval (c.i.) 1·09 to 1·69) for overall mortality and 1·26 (95 per cent c.i. 0·95 to 1·67) for cancer-specific mortality. Following palliative resection, there was no difference in survival between the affluent and deprived for either overall ($P = 0·27$) or cancer-specific ($P = 0·89$) mortality.

Conclusion: These findings confirm that the cancer-specific survival rate following surgery for colorectal cancer is lower in deprived patients. Stage of disease at presentation and type of operation did not account for this difference. The excess mortality was confined to patients undergoing apparently curative resection.

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Introduction

Colorectal cancer is the second commonest cause of cancer death in the westernized world. Survival is poor: in the UK, only one in three patients with colorectal cancer survives for 5 years[1]. Several studies of colorectal cancer from Europe and the USA have highlighted the variations in outcome for patients from different socioeconomic backgrounds[1–7]. Almost exclusively, the more deprived patients have lower survival rates. For example, in the UK, Coleman and colleagues[1] have reported a 5 per cent difference in relative survival in patients from the most deprived areas compared with the most affluent. There are a number of potential explanations for these observed differences in survival. These include delay in presentation, more advanced stage at the time of initial presentation, and variations in treatment. To date, no study has attempted to assess the impact of these factors on the socioeconomic differences in survival in patients with colorectal cancer.

Central Scotland has a wide spectrum of socioeconomic groups, often living in close proximity to one another, a reasonably homogeneous health service and a well established cancer registry. This makes Scotland an ideal setting to undertake a study to examine the distribution of established prognostic factors and assess the contribution of these factors to variations in outcome according to socioeconomic status.

Patients and methods

A total of 2269 patients who underwent resection for colorectal cancer between 1 January 1991 and 31 December 1994 in eight hospitals in the central belt of Scotland was included in the study. Information was abstracted from case

Table 1 Overall 5-year survival rates from the audit study (1991–1994) and the Scottish Cancer Registry (1986–1995) by deprivation category

	Affluent	Intermediate	Deprived	Difference*
Audit data (1991–1994)				
No. of patients	429	1442	398	
5-year survival rate (%)	41·7	38·9	36·7	5·0
Cancer registry data (1986–1995)				
No. of patients	5814	17 078	4861	
5-year survival rate (%)	35·0	32·5	30·3	4·7

*Affluent – deprived survival rate

notes by two specially trained data managers. Data for 1991 and 1992 were collected retrospectively and those for 1993 and 1994 prospectively. Details included mode of presentation, extent of tumour spread, surgical procedure, postoperative mortality, Dukes' stage and adjuvant therapy.

Patients were deemed to have had a curative resection if the surgeon considered that there was no macroscopic residual tumour once resection had been completed. Patients with distant metastases who underwent resection or in whom inadequate local clearance had been achieved were deemed to have had a palliative resection.

The extent of deprivation was defined using the Carstairs index[8]. This is an area-based measure derived from the 1991 census, using the postcode of residence at diagnosis, which divides the scores into a seven-point index. For illustrative purposes, the results are presented by amalgamating the seven categories into three groups: affluent (Carstairs categories 1 and 2), intermediate (categories 3, 4 and 5) and deprived (categories 6 and 7).

Date and cause of death were checked with data received by the cancer registration system through linkage with the Registrar General (Scotland). Deaths up to the end of 1999 have been included in the study.

Survival rates at 5 years were calculated using Kaplan–Meier estimates. Tests for trends in survival rates were carried out using the log rank test across the seven Carstairs categories. Hazard ratios were calculated using the affluent group as the baseline with adjustment for age and sex[9]. Comparisons of the associations between prognostic, treatment factors and socioeconomic status were made with the χ^2 statistic for trend. The impact contributed by the above factors to the survival difference between the socioeconomic groups was assessed by entering these factors into a Cox proportional hazards model to measure the extent of attenuation of the risk differences.

To ensure that the above analysis was representative of the Scottish population as a whole, the results obtained in the above audit study were compared with those of all 27 753 patients identified from the national cancer registry as having been diagnosed between 1986 and 1995 with cancer of the colon or rectum (International Classification of Diseases (ICD) 9, codes 153 and 154).

Table 2 Baseline characteristics of the audit population

	No. of patients
Socioeconomic status	
Affluent	429 (18·9)
Intermediate	1442 (63·6)
Deprived	398 (17·5)
Sex	
M	1117 (49·2)
F	1152 (50·8)
Age group (years)	
< 55	230 (10·1)
55–64	494 (21·8)
65–74	786 (34·6)
≥ 75	759 (33·5)
Mode of presentation	
Elective	1545 (68·1)
Emergency	724 (31·9)
Dukes' stage	
A	139 (6·1)
B	992 (43·7)
C	687 (30·3)
'D'	451 (19·9)
Type of resection	
Curative	1570 (69·2)
Palliative	699 (30·8)

Values in parentheses are percentages

Results

Overall 5-year survival rates for the 2269 patients undergoing resection between 1991 and 1994, and the corresponding overall survival rates for the Scottish population, are shown in *Table 1*. In the audit study, the overall survival difference between the affluent and deprived groups (5·0 per cent) was within the range of that seen in the registry data.

Of the 2269 patients included in the present audit, 17·5 per cent were socioeconomically deprived; 31·9 per cent presented as an emergency and 19·9 per cent had evidence of metastatic spread at the time of operation (*Table 2*). Some 1570 patients (69·2 per cent) underwent apparently curative resection and 699 (30·8 per cent) had a palliative resection.

There were 1523 deaths, 886 after curative resection; 51·6 per cent of patients undergoing apparently curative resection and 10·9 per cent of those undergoing palliative resection survived for 5 years. The cancer-specific survival rate at 5 years was 65·2 per cent after apparently curative resection and 14·3 per cent following palliative resection.

The distributions of the main prognostic factors known to influence survival in colorectal cancer are shown in

Tables 3 and *4*. There were no significant differences in mode of presentation, extent of disease, type of resection and postoperative mortality among the deprivation categories. Fewer deprived patients received adjuvant therapy.

Overall and cancer-specific survival at 5 years by deprivation category is shown in *Tables 5* and *6*. There were no significant differences in survival in terms of mode of presentation or Dukes' stage among the deprivation categories. In contrast, there were differences in relation to the type of operation. Following curative resection, the overall survival rate was 47·0 per cent in the deprived patients compared with 55·4 per cent in the affluent ($P = 0{\cdot}05$). The cancer-specific survival rate was 62·6 per cent in the deprived patients and 68·1 per cent in the affluent ($P = 0{\cdot}05$). This disadvantage was confined mainly to those undergoing elective surgery ($P = 0{\cdot}01$ and $P = 0{\cdot}03$ respectively). Following palliative resection there were no differences in survival between the affluent and the deprived ($P = 0{\cdot}27$ and $P = 0{\cdot}89$ respectively).

Overall and cancer-specific survival by deprivation category in patients undergoing curative resection adjusted for age, sex, mode of presentation and Dukes' stage is shown in *Table 7*. With increasing deprivation there was a significant trend towards poorer overall ($P = 0{\cdot}001$) and cancer-specific ($P = 0{\cdot}02$) survival. Taking the affluent as the baseline, the adjusted hazard ratio for overall survival for deprived patients undergoing curative resection was 1·36 (95 per cent confidence interval (c.i.) 1·09 to 1·69); the corresponding adjusted hazard ratio for cancer-specific survival was 1·26 (95 per cent c.i. 0·95 to 1·67). In patients undergoing palliative resection, there was no significant trend across the deprivation categories for either overall survival ($P = 0{\cdot}57$) or cancer-specific survival ($P = 0{\cdot}71$); the corresponding adjusted hazard ratios for the deprived relative to the affluent were 1·13 (95 per cent c.i. 0·87 to 1·47) and 1·09 (95 per cent c.i. 0·82 to 1·45) respectively.

Table 3 Characteristics of patients from affluent, intermediate and deprived areas

	Affluent	Intermediate	Deprived	*P**
Sex				
M	219 (51·0)	702 (48·7)	196 (49·2)	0·31
F	210 (49·0)	740 (51·3)	202 (50·8)	
Age group				
< 55	31 (7·2)	155 (10·7)	44 (11·1)	0·19
55–64	109 (25·4)	306 (21·2)	79 (19·8)	
65–74	129 (30·1)	505 (35·0)	152 (38·2)	
≥ 75	160 (37·3)	476 (33·0)	123 (30·9)	
Mode of presentation				
Elective	294 (68·5)	978 (67·8)	273 (68·6)	0·80
Emergency	135 (31·5)	464 (32·2)	125 (31·4)	
Dukes' stage				
A	33 (7·7)	90 (6·2)	16 (4·0)	0·85
B	166 (38·7)	647 (44·9)	179 (45·0)	
C	141 (32·9)	424 (29·4)	122 (30·7)	
'D'	89 (20·7)	281 (19·5)	81 (20·4)	

Values in parentheses are percentages. *Derived from a test for trend across the original seven Carstairs categories

Discussion

In this audit study, in which survival was representative of the Scottish population as a whole, it was confirmed that, compared with those from more affluent areas, deprived patients presenting with colorectal cancer have poorer overall and cancer-specific survival rates. Furthermore, this survival disadvantage is confined mainly to patients undergoing apparently curative resection, the excess risk of the deprived patients dying being 36·0 per cent (*Table 7*; hazard ratio 1·36). There was no difference in survival between affluent and deprived patients following palliative resection.

Previously, it has been suggested that these differences might be due to more advanced disease at initial presentation or to variations in treatment. However, this study has shown that there were no significant differences in mode of presentation, extent of disease, the proportion of patients who underwent curative resection or postoperative mortality between the deprived and affluent patients. Fewer deprived patients received adjuvant therapy, but this had no effect on survival as the overall proportion of patients receiving adjuvant therapy at the time of the study was low. A similar difference in the proportion of patients receiving adjuvant chemotherapy according to socioeconomic status has been observed previously[10].

These results differ from those of previous studies. A study from south-east England[11] showed marked variation in the rate of emergency admissions between affluent and deprived patients. The difference between the two studies may be due to variations in the definition of what constitutes an emergency admission and in the organization of the

Table 4 Type of operation, postoperative mortality and adjuvant treatment by deprivation category

	Affluent	Intermediate	Deprived	*P*†
Type of resection				
Curative	298 (69·5)	985 (68·3)	287 (72·1)	0·52
Palliative	131 (30·5)	457 (31·7)	111 (27·9)	
30-day mortality				
Curative	10 (3·4)	45 (4·6)	12 (4·2)	0·41
Palliative	13 (9·9)	42 (9·2)	13 (11·7)	0·98
Adjuvant therapy*	16 (5·4)	36 (3·7)	5 (1·7)	0·01

Values in parentheses are percentages. *Curative resections. †Derived from a test for trend across the original seven Carstairs categories relevant services. A pathology-based study from east Scotland reported that deprived patients were more likely to present with more advanced local disease[12]. However, that analysis was based on a relatively small cohort; it would also appear that no cognizance was taken of whether or not the patient had metastatic disease. In neither of the above studies was the impact of the observed differences on survival assessed.

The reason why deprived patients are at increased risk of dying from colorectal cancer following apparently curative resection is not clear. However, from this study it would appear that these differences in survival between the affluent and deprived are not due solely to the intrinsic properties of the primary tumour because neither stage of disease at presentation nor variations in treatment were responsible. An alternative explanation for the effect of deprivation on survival may be that the tumour–host response is compromised in the deprived patients. It is known that up to one-third of patients undergoing apparently curative resection harbour 'occult' hepatic metastases at the time of operation and it is the presence or absence of these occult metastases that largely determines the likelihood of dying from disseminated disease[13]. It may be that there is some ongoing process that compromises the ability of the host to mount a response against these micrometastases. Such processes might include impaired immunological function as a result of poor nutrition or the continuing presence of a systemic inflammatory response, both of which have been shown to be associated with poor outcome in patients with cancer[14–17].

Table 5 Five-year overall survival by deprivation category

	5-year survival rate (%)			
	Affluent	Intermediate	Deprived	P*
Mode of presentation				
Emergency	29·6	27·8	23·2	0·86
Elective	47·3	44·2	42·8	0·15
Dukes' stage				
A	84·9	73·3	56·3	0·06
B	55·4	54·4	54·7	0·32
C	36·9	28·1	27·1	0·18
'D'	7·9	8·5	7·4	0·69
Type of resection				
Overall				
Curative	55·4	51·8	47·0	0·05
Palliative	10·7	11·2	9·9	0·27
Emergency				
Curative	39·5	39·4	33·3	0·92
Palliative	12·2	6·2	8·9	0·77
Elective				
Curative	61·8	57·2	51·9	0·01
Palliative	9·8	13·9	11·5	0·37

*Derived from a test for trend across the original seven Carstairs categories

Table 6 Five-year cancer-specific survival by deprivation category

	5-year survival rate (%)			
	Affluent	Intermediate	Deprived	P*
Mode of presentation				
Emergency	36·6	38·4	35·8	0·37
Elective	58·0	54·2	55·0	0·28
Dukes' stage				
A	90·3	89·1	79·0	0·71
B	70·9	68·4	71·5	0·34
C	44·7	36·1	36·3	0·20
'D'	10·8	10·8	10·8	0·86
Type of resection				
Overall				
Curative	68·1	65·0	62·6	0·05
Palliative	13·4	14·8	13·4	0·89
Emergency				
Curative	50·0	53·0	49·1	0·26
Palliative	13·8	11·0	14·0	0·73
Elective				
Curative	75·1	70·1	67·5	0·03
Palliative	13·3	17·0	12·8	0·81

*Derived from a test for trend across the original seven Carstairs categories

Table 7 Hazard ratios in patients undergoing curative resection by deprivation category

Factors adjusted for in model	Affluent	Intermediate	Deprived	P*
Overall deaths				
Sex and age	1	1·20 (1·00, 1·44)	1·36 (1·08, 1·67)	0·002
Sex, age, mode of presentation and Dukes' stage	1	1·23 (1·03, 1·47)	1·36 (1·09, 1·69)	0·001
Tumour-specific deaths				
Sex and age	1	1·17 (0·93, 1·47)	1·24 (0·94, 1·64)	0·05
Sex, age, mode of presentation and Dukes' stage	1	1·23 (0·98, 1·54)	1·26 (0·95, 1·67)	0·02

Values in parentheses are 95 per cent confidence intervals. *Derived from a test for trend across the original seven Carstairs categories

In conclusion, this study has confirmed previous reports that, compared with more affluent patients, deprived patients have poorer survival following surgery for colorectal cancer. This disadvantage appears to be confined to those undergoing apparently curative resection. These differences in outcome do not appear to be due to tumour-related factors including mode of presentation, extent of disease, type of operation, postoperative mortality or adjuvant treatment. It may be, therefore, that these differences in outcome between the deprived and affluent patients are due to other factors, including a compromised tumour–host response.

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Original article

Prospective study of the incidence and outcome of intra-abdominal hypertension and the abdominal compartment syndrome

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Background: Intra-abdominal hypertension has been recognized as a source of morbidity and mortality in the traumatized patient following laparotomy. Multiple organ dysfunction attributable to intra-abdominal hypertension has been called the abdominal compartment syndrome. The epidemiology and characteristics of these processes remain poorly defined.

Methods: Intra-abdominal pressure was measured prospectively in all patients admitted to a trauma intensive care unit over 9 months. Data were gathered on all patients with intra-abdominal hypertension.

Results: Some 706 patients were evaluated. Fifteen (2 per cent) of 706 patients had intra-abdominal hypertension. Six of the 15 patients with intra-abdominal hypertension had abdominal compartment syndrome. Half of the patients with abdominal compartment syndrome died, as did two of the remaining nine patients with intra-abdominal hypertension. Patients with abdominal compartment syndrome had a mean intra-abdominal pressure of 42 mmHg compared with 26 mmHg in patients with intra-abdominal hypertension only ($P < 0.05$).

Conclusion: The incidence of intra-abdominal hypertension and abdominal compartment syndrome was 2 and 1 per cent respectively. Intra-abdominal hypertension did not necessarily lead to abdominal compartment syndrome, and often resolved without clinical sequelae. Abdominal compartment syndrome did not occur in the absence of earlier laparotomy. Abdominal compartment syndrome was associated with a marked increase in intra-abdominal pressure (above 40 mmHg).

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Introduction

Intra-abdominal hypertension, defined as a sustained increase in intra-abdominal pressure, can cause direct mechanical impairment of respiratory, haemodynamic, renal and splanchnic function. Organ dysfunction attributable to increased intra-abdominal pressure has been termed the abdominal compartment syndrome. The abdominal compartment syndrome has been increasingly recognized as a source of morbidity and mortality in the traumatized patient following laparotomy, typically as the result of massive visceral oedema, retroperitoneal haemorrhage or intra-abdominal packing[1–5]. Abdominal compartment syndrome has also been reported in non-traumatized patients[6–11].

Despite several recent reviews[12–14], fundamental questions about intra-abdominal hypertension and abdominal compartment syndrome remain unanswered. The overall incidence of intra-abdominal hypertension and abdominal compartment syndrome, the importance of an isolated finding of raised intra-abdominal pressure, the clinical progression of intra-abdominal hypertension, and the level of increased pressure that is associated with clinically significant impairment of respiratory, haemodynamic, renal and splanchnic function are all unknown. This study measured intra-abdominal pressure prospectively in a cohort of patients to characterize better the clinical significance of abdominal compartment syndrome and intra-abdominal hypertension in critically ill trauma patients. The ultimate goal of the study was to define a specific set of risk factors that would allow early identification of patients at high risk of developing abdominal compartment syndrome, ideally before overt manifestation of multiple organ dysfunction.

Patients and methods

From April 1998 to January 1999, all patients admitted to the intensive care unit (ICU) at a level I trauma centre underwent serial indirect measurement of intra-abdominal pressure via an indwelling urinary catheter. The standard procedure used was that first popularized by Kron *et al.*[6] and subsequently validated clinically[15–17]. Measurements were obtained initially at 6-h intervals. The study protocol was approved by the Institutional Review Board at the University of Miami/Jackson Memorial Hospital.

If intra-abdominal pressure remained below 20 mmHg without evidence of organ system dysfunction, measurements were discontinued after 24 h. If it was 20 mmHg or higher and was sustained for two reproducible measurements, the patient was given a diagnosis of intra-abdominal hypertension, and additional data were collected: haemodynamic variables (heart rate, blood pressure, central venous pressure and right heart catheter data if available); respiratory variables (respiratory rate, ventilator settings if applicable); renal variables (urine output, serum creatinine and blood urea nitrogen); Injury Severity Score (ISS); and the amount and composition of fluid resuscitation before and after the increased intra-abdominal pressure was initially recorded. Intra-abdominal pressure measurements were also increased in frequency from every 6 h to every 1–4 h. If intra-abdominal hypertension was associated with multiple organ dysfunction syndrome (MODS), abdominal compartment syndrome was suspected. If clinical suspicion was high, a laparotomy for abdominal decompression was performed. The diagnosis of abdominal compartment syndrome was ultimately confirmed when physiological variables improved with decompressive laparotomy.

Statistical analysis was performed using Fisher's exact test.

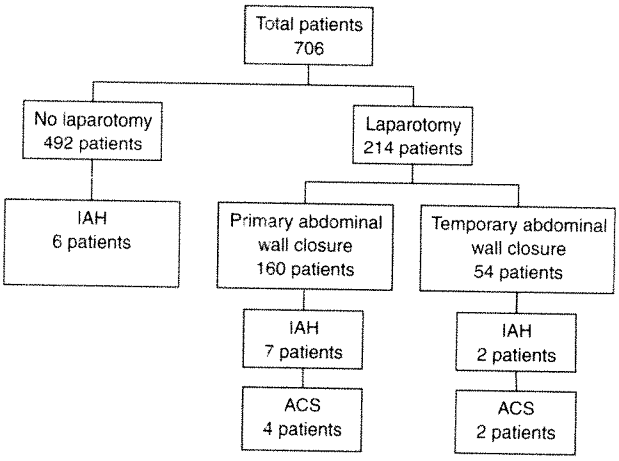
Total patients 706
No laparotomy 492 patients
Laparotomy 214 patients
IAH 6 patients
Primary abdominal wall closure 160 patients
Temporary abdominal wall closure 54 patients
IAH 7 patients
IAH 2 patients
ACS 4 patients
ACS 2 patients

Fig. 1 Intra-abdominal hypertension (IAH) and compartment syndrome (ACS) in patients admitted to a trauma intensive care unit

Table 1 Characteristics of trauma intensive care unit patient population

Mean (range) age (years)	42 (14–90)
Sex ratio (M : F)	508 : 198
Mean (range) Injury Severity Score	18 (1–75)
Deaths*	88 (12)
Mechanism of injury*	
Motor vehicle crash	311 (44)
Gunshot wound	155 (22)
Pedestrian/cyclist struck	107 (15)
Stab wound	49 (7)
Fall	42 (6)
Assault	21 (3)
Other†	21 (3)

*Values in parentheses are percentages. †For example, boating accident, mauling, train injury

Results

The findings of the study are summarized in *Fig. 1*. A total of 706 patients was evaluated during the study period. The characteristics of these patients are summarized in *Table 1*. Fifteen (2 per cent) of 706 patients had intra-abdominal hypertension, defined as sustained intra-abdominal pressure of 20 mmHg or more. Six of these 15 patients had abdominal compartment syndrome confirmed at decompressive laparotomy (1 per cent of total admissions). The characteristics of these 15 patients are summarized in *Table 2*.

Table 3 summarizes notable differences between patients with abdominal compartment syndrome and those with intra-abdominal hypertension only. The six patients with abdominal compartment syndrome had a mean(s.d.) ISS of 34(12) and an intra-abdominal pressure of 42(11) mmHg. Three of the six patients died from progressive multiple organ failure despite abdominal decompression. Notably, two of the six patients developed abdominal compartment syndrome despite 'prophylactic' temporary abdominal closure.

The nine patients with intra-abdominal hypertension who did not undergo abdominal decompression had a mean(s.d.) ISS of 27(12) and a mean(s.d.) intra-abdominal pressure of 26(4) mmHg. Two of these nine patients died, one from a late pulmonary embolus during rehabilitation after discharge from the ICU and one from complications of a severe closed head injury. Four of the nine patients had intra-abdominal hypertension without laparotomy before admission. Two of these patients had resolution of intra-

Table 2 Characteristics of patients with intra-abdominal hypertension and abdominal compartment syndrome

	Intra-abdominal hypertension only	Abdominal compartment syndrome
Sex ratio (M : F)	9 : 0	4 : 2
Mean (range) age (years)	42 (23–76)	44 (21–88)
Cause of injury		
Gunshot wound	4	2
Motor vehicle crash	4	2
Pedestrian struck	1	1
Fall		1
Type of injury		
Isolated vascular injury	1	
Retroperitoneal haematoma, amputation	1	
Multiple thoracic injuries	1	
Multiple abdominal injuries	2	1
Multiple abdominal and orthopaedic injuries	2	3
Multiple abdominal and thoracic injuries		2
Multiple abdominal, thoracic and head injuries	1	
Multiple abdominal, thoracic and orthopaedic injuries	1	
Clinical course		
Abdominal decompression		6
Discharged from hospital	7	3
Deaths	2	3
Head injury	1	
Pulmonary embolus	1	
Multiple organ dysfunction syndrome		3

Table 3 Comparison of intra-abdominal pressure, Injury Severity Score and mortality rate in patients with intra-abdominal hypertension and abdominal compartment syndrome

	Intra-abdominal hypertension only	Abdominal compartment syndrome	P^*
Intra-abdominal pressure (mmHg)	26(4)	42(11)	< 0·05
Injury Severity Score	27(12)	34(12)	0·08
Mortality rate	2 of 9	3 of 6	0·07

Values are mean(s.d.). *Fisher's exact test

abdominal hypertension following 'large' bowel movements.

There were trends toward increased ISS and increased mortality rate in the patients with abdominal compartment syndrome. However, only the difference in intra-abdominal pressure between the two groups reached statistical significance: 26(4) *versus* 42(11) mmHg ($P < 0{\cdot}05$).

Discussion

Although MODS has been described for over 30 years, its treatment remains primarily supportive. The abdominal compartment syndrome – like tension pneumothorax, its better known analogue in the chest – is a potentially treatable cause of early MODS in the severely injured patient. Like tension pneumothorax, abdominal compartment syndrome causes organ dysfunction by direct mechanical effects. Respiratory derangement occurs as the result of elevation of the diaphragm, causing decreased functional residual capacity and increases in airway pressures in patients on positive pressure ventilation[18]. Haemodynamic derangements are the result of decreased cardiac preload from compression of the inferior vena cava with resultant decreased cardiac output[19]. Compression of the renal vein results in impairment in kidney function[20]. Increased intra-abdominal pressure has also been shown to decrease splanchnic perfusion[21,22]. Once diagnosed, abdominal compartment syndrome requires and responds immediately to invasive therapeutic intervention.

That this diagnosis may be a difficult one to make further complicates an already complex clinical problem. Among trauma surgeons abdominal compartment syndrome is a well established diagnosis[23]. However, despite numerous series in the literature, much about abdominal compartment syndrome remains unknown and therefore controversial, in part because of confusion regarding terminology. There are, as yet, no universally accepted definitions. This study used a broad definition of intra-abdominal hypertension to ensure capture of all patients with a sustained increase in intra-abdominal pressure. The threshold for intra-abdominal hypertension was defined arbitrarily as a sustained intra-abdominal pressure of 20 mmHg and above.

Normal intra-abdominal pressure is highly variable in healthy individuals, depending on body position, body habitus and activity. Transient increases in intra-abdominal pressure to 80 mmHg have been described in normal subjects[24–26]. Sustained pneumoperitoneum to 15 mmHg is routinely used without apparent adverse effects during laparoscopic surgery[27]. In the present study 20 mmHg was chosen so that all individuals with even mild, occult intra-abdominal hypertension were included and followed, with the goal of characterizing the spectrum of disease, from a mild asymptomatic pressure increase to frank abdominal compartment syndrome, and to ensure that all cases of intra-abdominal hypertension were captured. In the absence of baseline data of this kind, no true estimate of the overall incidence of intra-abdominal hypertension and abdominal compartment syndrome may be made.

For the purposes of this study, abdominal compartment syndrome was narrowly defined as intra-abdominal hypertension with associated MODS that improved following abdominal decompression. This very strict definition of abdominal compartment syndrome was used to ensure that the diagnosis of abdominal compartment syndrome was accurate. Some patients with intra-abdominal hypertension associated with relatively mild organ dysfunction may not require decompressive laparotomy, but will respond with non-operative observation; according to the present definitions, such patients had intra-abdominal hypertension and not true abdominal compartment syndrome.

The ultimate goal of this study was to identify specific risk factors that would allow early diagnosis of intra-abdominal hypertension before the onset of frank MODS, for example the amount and composition of fluid resuscitation, specific injuries such as pelvic fracture or hepatic injury, the use of perihepatic packing, etc. However, the incidence of intra-abdominal hypertension and abdominal compartment syndrome in this cohort of patients was surprisingly low, and did not allow a statistically meaningful analysis of risk factors.

The data show that intra-abdominal hypertension and abdominal compartment syndrome are rare (2 and 1 per cent respectively). This differs markedly from two previous series, which reported incidences of 14 per cent[28] and 33 per cent[29]. There are two plausible explanations for this discrepancy. First, by measuring intra-abdominal pressure in all patients admitted to the ICU, this study included low-risk as well as high-risk patients, whereas the previous studies confined data collection to high-risk patients. While the latter approach ensures a good yield of patients with abdominal compartment syndrome, it may result in a very high incidence compared with that seen clinically in the ICU population overall. Furthermore, such an approach potentially misses patients who are not at high risk, and yet may have MODS falsely attributed to sepsis or irreversible shock when in fact they have unrecognized abdominal compartment syndrome. By measuring intra-abdominal pressure prospectively in all patients, this study obtained a true overall incidence. By using this methodology, the study also identified all cases of occult intra-abdominal hypertension, regardless of whether they were associated with clinical sequelae.

The second reason for such a low incidence is the large number of temporary abdominal closures performed; 54 (25 per cent) of 214 patients who underwent laparotomy had temporary abdominal closure before arrival in the ICU, and before any measurement of intra-abdominal pressure. Some characteristics of these patients are shown in *Table 4*. In most cases temporary abdominal closure was performed using a prosthetic mesh device or a 'Bogota' bag to expand the abdominal wall, as prophylaxis against the development of abdominal compartment syndrome. If even a small number of these patients had undergone primary fascial closure, the incidence of intra-abdominal hypertension and abdominal compartment syndrome may have been substantially higher.

Table 4 Characteristics of 54 patients who had temporary abdominal closure

Type of closure†	
Prosthetic mesh/'Bogota' bag‡	49 (2)
Primary closure of skin only§	5 (0)
Mean (range) age (years)	33 (19–67)
Sex ratio (M : F)	40 : 14
Mean (range) Injury Severity Score	31 (16–59)
Deaths*	21 (39)
Mechanism of injury*	
Motor vehicle crash	17 (31)
Gunshot wound	33 (61)
Pedestrian/cyclist struck	2 (4)
Stab wound	2 (4)

Values in parentheses are *percentages or †number of patients with abdominal compartment syndrome. ‡Allows expansion of abdominal wall for prophylaxis against abdominal compartment syndrome; §fascia left open, for planned re-exploration

The presence of intra-abdominal hypertension following temporary abdominal closure was an ominous finding. Two patients developed intra-abdominal hypertension despite prophylactic temporary abdominal closure, both of whom had frank abdominal compartment syndrome. Both patients required abdominal decompression, with the use of larger prosthetic materials to expand the abdominal cavity.

Similarly, the presence of intra-abdominal hypertension following laparotomy with primary fascial closure may be an ominous finding, suggestive of abdominal compartment syndrome. In this study intra-abdominal hypertension occurred in only 4 per cent (nine of 160) of patients following laparotomy and primary fascial closure. However, four of these patients had abdominal compartment syndrome as a result of intra-abdominal hypertension.

Abdominal compartment syndrome did not occur in the absence of an earlier laparotomy in the present study. There are increasing reports of abdominal compartment syndrome occurring without laparotomy in the literature, in burn patients (especially those with circumferential full-thickness torso burns) and in patients without intra-abdominal injury[30,31].

Intra-abdominal hypertension does not inevitably progress to abdominal compartment syndrome, and may be associated with relatively benign conditions, such as constipation. Two patients had resolution of intra-abdominal hypertension following 'large' bowel movements. An isolated finding of intra-abdominal hypertension in an otherwise asymptomatic patient is clearly different from frank abdominal compartment syndrome in a patient with severe abdominal trauma and worsening MODS.

In general, asymptomatic intra-abdominal hypertension had a benign clinical course. True abdominal compartment syndrome, by comparison, is associated with significant morbidity and mortality. Three of six patients with abdominal compartment syndrome died from progressive MODS, despite early recognition and abdominal decompression.

Abdominal compartment syndrome was also associated with a significantly higher intra-abdominal pressure than asymptomatic intra-abdominal hypertension (42 *versus* 26 mmHg). Although not unexpected, this is a meaningful finding; an intra-abdominal pressure of 40 mmHg clearly is more worrisome than one of 20 mmHg, especially when associated with MODS.

Based on the findings of this study, routine measurement of intra-abdominal pressure is not necessary in all patients, given its rarity and given the benign nature of asymptomatic intra-abdominal hypertension. However, routine measurement of intra-abdominal pressure is warranted in patients at high risk, certainly in those with early MODS following trauma, even if they have undergone prophylactic temporary abdominal closure previously. Because of the small number of patients with intra-abdominal hypertension and abdominal compartment syndrome, this study was not able to elucidate definitively variables that would characterize low or high risk.

Acknowledgements

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Original article

Prediction of the response to chemoradiation and prognosis in oesophageal squamous cancer

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Background: The sensitivity of cancer cells to chemotherapy and radiation therapy depends on various biological properties. This study investigated the expression of p53, CDC25B and metallothionein (MT), and evaluated their clinical significance in chemoradiation therapy (CRT) for oesophageal squamous cell carcinoma.

Methods: The expression of p53, CDC25B and MT was evaluated by immunohistochemistry using biopsy specimens taken before CRT for 77 patients with oesophageal squamous cell carcinoma, and correlated with the pathological effects of CRT and survival.

Results: p53-positive tumours and MT-positive tumours had a poor response to CRT, whereas tumours with strong CDC25B expression were associated with a good response. When each patient was scored for the presence of the three biological factors, there was a strong correlation between the sensitivity score and the pathological effect of CRT ($P < 0{\cdot}001$), and a (non-significant) difference in the 5-year survival rate between patients with a high score and those with a low score (67 *versus* 34 per cent respectively; $P = 0{\cdot}12$).

Conclusion: The combined evaluation of p53, CDC25B and MT may help to identify patients with advanced oesophageal squamous cell carcinoma who will benefit from preoperative CRT.

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Introduction

Chemoradiation therapy (CRT) is one of the most commonly used modalities for oesophageal cancer. Preoperative CRT using cisplatin and 5-fluorouracil has been used by the authors for advanced oesophageal cancer since 1989 to improve operative resectability and prognosis. Although the response rate to CRT using this protocol is high (64 per cent)[1], there is no survival benefit for non-responders. It would be useful to be able to predict the response to CRT so that non-responders could avoid side-effects; this has not been possible using the clinicopathological factors examined routinely.

One possible solution is offered by recent advances in tumour biology, especially concerning apoptosis. Apoptosis induced by CRT involves various biological phenomena such as DNA repair, altered drug metabolism, inflammation, molecular chaperoning and changes to the cell cycle. Various biological markers have been investigated for their association with a response to CRT, including metallothionein (MT), glutamine S transferase (GST)-pai, heat shock protein (HSP) 27, HSP-70, NF (nuclear factor) κB, I (inhibitory) κB, bcl-2, CDC25A, CDC25B, p53, cyclin D1, cyclin B1, Ki-67 and p21WAF1.

p53 is a tumour suppressor gene that contributes to the preservation of genetic stability by facilitating both G1 arrest and apoptosis in response to DNA damage[2,3]. Many studies have shown that apoptosis following DNA damage is inhibited by *p53* mutation and restored by wild-type *p53*[4]. *p53* abnormality is observed in more than 70 per cent of advanced oesophageal cancers[5]. Although *p53* may play a critical role in radiation-induced apoptosis[3], the discrepancy between the low frequency of normal p53 function and the high clinical response of oesophageal cancer strongly suggests the existence of an alternative pathway, independent of p53, that regulates radiation sensitivity. The G2–M checkpoint, which is well preserved regardless of *p53* status, is strongly associated with radiation-induced apoptosis[6]. In previous studies, it was found that CDC25B, a dual phosphatase that activates CDC2 and the G2–M progression[7,8], was significantly associated with radiation sensitivity among various molecules regulating the G2–M checkpoint[9]. Metallothionein (MT) is an intracellular metal-binding protein involved in zinc homoeostasis and the detoxification of heavy metals[10]. Experimentally, MT affects cisplatin-induced apoptosis[11], and a positive association has also been identified between MT expression and resistance to CRT[12].

In the present study an attempt was made to predict the effect of CRT by evaluating the expression of p53, CDC25B and MT simultaneously. Their influence on the clinical outcome of this multimodal therapy was also examined.

Patients and methods

Seventy-seven patients with advanced oesophageal squamous cell carcinoma who underwent CRT followed by oesophagectomy at Osaka University Medical School from 1989 to 2000 were enrolled in the study. There were 67 men and ten women of mean age 59·5 (range 37–83) years. The diagnosis of squamous cell carcinoma was confirmed histologically from biopsy specimens obtained before starting CRT.

All patients underwent attempted curative operation (R_0), including subtotal oesophagectomy and regional lymph node resection, 4–6 weeks after completing CRT. The entire oesophageal specimen was sliced into serial 5-mm longitudinal sections and examined histologically for the response to CRT, according to the following criteria: grade 1, a minor effect with viable cancer cells accounting for more than one-third of the tumour tissue; grade 2, a moderate effect with viable cancer cells accounting for less than one-third of the tumour tissue; and grade 3, no residual viable cancer cells[13].

Chemoradiation therapy

A total radiation dose of 40 Gy was applied; 2-Gy fractions were delivered daily for 4 weeks by a 10-MV linear accelerator to the mediastinum and the neck. Concurrent chemotherapy consisted of daily administration of cisplatin 7–10 mg/m^2 by intravenous infusion for 2 h and 5-fluorouracil 350–500 mg/m^2 as a continuous intravenous infusion for 24 h. The therapy was administered 5 days per week for 4 weeks, completely synchronized with the irradiation[1].

Immunohistological staining procedure and evaluation of staining

Immunostaining was performed using streptavidin–peroxidase complex methods for MT and the EnVision+™ peroxidase kit (DAKO, Glostrup, Denmark)[14] on a TeckMate Horizon automated staining system (DAKO, Glostrup, Denmark) for p53 and CDC25B, as described previously[15]. Primary antibodies were incubated overnight at 4°C with E9 (dilution 1 : 50; DAKO, Kyoto, Japan) for MT, and for 1 h at room temperature with DO-7 (dilution 1 : 50; Novocastra Laboratories, Newcastle upon Tyne, UK) for p53 and with C45820 (5 µg/ml; Transduction Laboratories, Lexington, Kentucky, USA) for CDC25B.

The immunoreactivity of CDC25B was classified according to the frequency of the positively stained cells as follows: negative (–), less than 10 per cent; positive (+), 10–50 per cent; overexpressed (+ +), more than 50 per cent. For p53 and MT staining, when more than 10 per cent of the cancer cells showed positive staining, the tumours were evaluated as positive (+) (*Fig. 1*). In previous studies[9,12] these cut-off values yielded the best gradation in correlation with the histological response to CRT. As the amount of biopsy material was limited, it was not possible to evaluate all three molecules in some patients. p53 was evaluated in all 77 tumours, MT in 75 and CDC25B in 68. All three molecules were scored in 66 tumours. As each p53(–), MT (–) and CDC25B(+ +) pattern showed a higher CRT response than its counterpart, CRT sensitivity was scored as the number of these high-sensitivity subgroups. Thus, the tumours were classified into four groups with scores of 0–3 by the addition of points: p53(+), MT(+) and CDC25B(–)/(+), each of which scored 0; and p53(–), MT(–) and CDC25B(+ +), each of which scored 1.

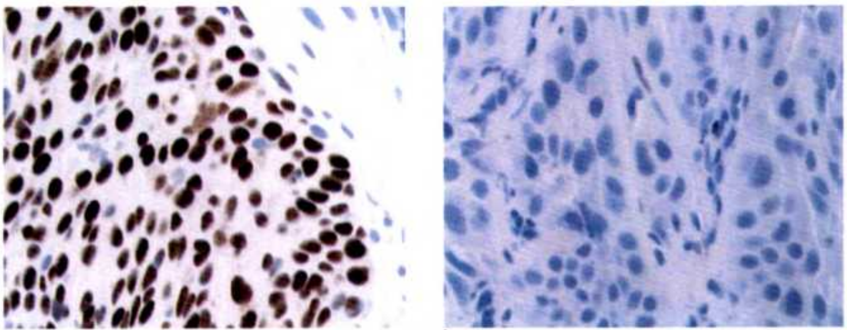
Statistical analysis

Associations between two parameters were analysed with the Mann–Whitney *U* test or Spearman's rank correlation test. For continuous parameters, the difference between the two groups was analysed with Student's *t* test. Multivariate analysis with a logistic regression model was used to determine the independent significant parameters to predict the histological effects of CRT. Postoperative survival was analysed using the Kaplan–Meier method and assessed statistically by means of the log rank test. For all analyses $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the software package StatView version 5·0 (Abacus Concepts, Berkeley, California, USA).

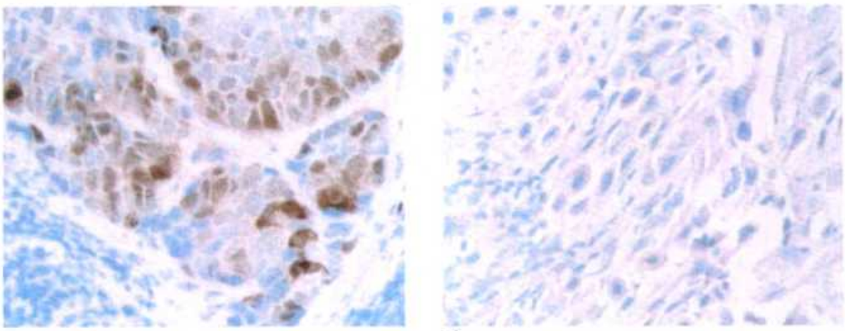
Results

Correlation between histological response to chemoradiation and prognosis

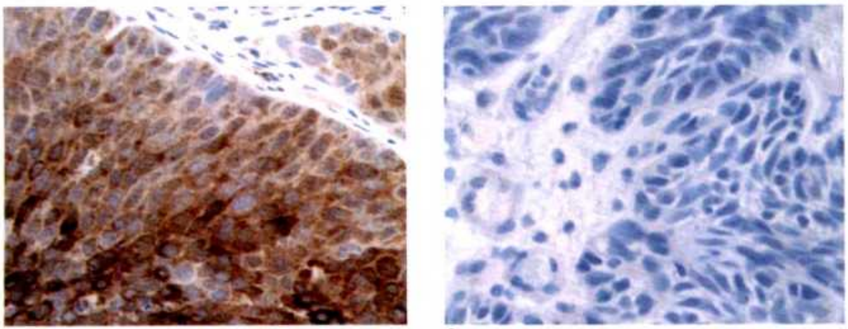
The histological response of the 77 patients to CRT was classified as follows, according to the volume of residual



a p53 positive **b** p53 negative



c MT positive **d** MT negative



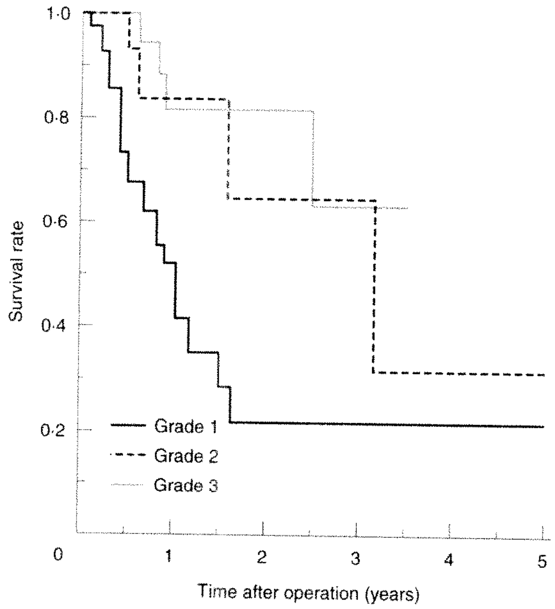
e CDC25B positive **f** CDC25B negative

Fig. 1 Immunohistochemical expression of p53, metallothionein (MT) and CDC25B. Oesophageal cancers were classified by nuclear p53 expression as positive (**a**) or negative (**b**), according to the frequency of stained cells. MT and CDC25B were detected in the nucleus and/or cytoplasm, and classified as positive (MT (**c**) and CDC25B (**e**)) or negative (MT (**d**) and CDC25B (**f**)) (original magnification × 400)

tumour cells: grade 1, 41 (53 per cent); grade 2, 17 (22 per cent); and grade 3, 19 (25 per cent). There was a significant association between the histological response to CRT and prognosis (*Fig. 2*), but not with any clinicopathological variables before starting CRT, including age, sex, tumour location, tumour length and histological differentiation (data not shown).

Relationship of p53, CDC25B and metallothionein with histological response to chemoradiation therapy and clinical outcome

These molecules were expressed in squamous cell carcinomas of the oesophagus, characteristically localized in the nucleus for p53, and in both the nucleus and cytoplasm for



No. at risk						
Grade 1	41	15	5	5	1	0
Grade 2	17	9	5	2	0	0
Grade 3	19	12	10	5	4	3

Fig. 2 Kaplan–Meier survival curves classified by histological response to chemoradiation therapy. $P = 0{\cdot}017$ (grade 1 *versus* grade 2), $P = 0{\cdot}001$ (grade 1 *versus* grade 3) (log rank test)

MT and CDC25B (*Fig. 1*). Using biopsy specimens before CRT, tumours were classified as positive (+) for p53 and MT in 47 (61 per cent) of 77 patients and in 43 (57 per cent) of 75 patients respectively. The CDC25B expression pattern was classified, as reported previously[9], into: overexpressed (+ +), 14 (21 per cent) of 68 tumours; positive (+), 20 (29 per cent) of 68; and negative (–), 34 (50 per cent) of 68 (*Table 1*).

The response to CRT was correlated with the expression pattern of the three biological markers (*Table 1*). There was a significant inverse correlation between p53 status and the response to CRT ($P = 0{\cdot}005$), which was grade 3 in almost half (14 of 30) of p53(–) tumours, but in only five of 47 p53(+) tumours. Tumours expressing MT were significantly more resistant to CRT ($P = 0{\cdot}033$). There was also a positive correlation between CDC25B expression and the response to CRT: a grade 3 response was observed in seven of 14 CDC25B(+ +) and seven of 34 CDC25B(–) tumours ($P = 0{\cdot}038$).

The 5-year survival rates of patients with p53(+) and p53(–) tumours were 37 and 27 per cent respectively ($P = 0{\cdot}40$); similar rates were observed for MT(+) and MT(–) tumours (40 *versus* 22 per cent; $P = 1{\cdot}0$). There was a tendency for patients with tumours having CDC25B(+ +) to show better survival than those with CDC25B(+) or (–) tumours, although the difference was not statistically significant (5-year survival rate 59 and 34 per cent respectively; $P = 0{\cdot}27$).

Table 1 Correlation of p53, metallothionein and CDC25B expression with histological response to chemoradiation

	Response to chemoradiation				
	Grade 1	Grade 2	Grade 3	Total	*P**
p53					0·005
(+)	28	14	5	47 (61)	
(–)	13	3	14	30 (39)	
Total	41	17	19	77 (100)	
Metallothionein					0·033
(+)	29	5	9	43 (57)	
(–)	12	11	9	32 (43)	
Total	41	16	18	75 (100)	
CDC25B					0·038
(–)	20	7	7	34 (51)	
(+)	11	5	4	20 (29)	
(+ +)	3	4	7	14 (21)	
Total	34	16	18	68 (100)	

Values in parentheses are percentages. *Spearman's rank correlation test

Prediction of the response to chemoradiation therapy and clinical outcome using three biological markers

There was no significant correlation among the expression of three biological markers in the rank correlation test (p53 *versus* MT, $P = 0{\cdot}46$; p53 *versus* CDC25B, $P = 0{\cdot}17$; MT *versus* CDC25B, $P = 0{\cdot}91$). Multivariate analysis using logistic regression of these three markers revealed that the p53 status was a significant parameter for discriminating a grade 3 response to CRT from grades 1 and 2. To discriminate a grade 1 response from grades 2 and 3, the statuses of MT, p53 and CDC25B were all significant (hazard ratio 5·77, $P = 0{\cdot}005$; hazard ratio 3·84, $P = 0{\cdot}034$; hazard ratio 4·82, $P = 0{\cdot}045$ respectively).

Next, the biological response to CRT was scored from 0 to 3 according to the number of sensitive subgroups, that is, negativity for p53 and MT and overexpression of CDC25B (*Table 2*). As a result, 19 (29 per cent) of 66 tumours were classified as score 0, 32 (48 per cent) of 66 as score 1, 13 (20 per cent) of 66 as score 2, and two (3 per cent) of 66 as score 3. The correlation between the biological CRT sensitivity score and the histological response to CRT was statistically significant ($P < 0{\cdot}001$). For example, 15 of 19 score 0 tumours and 17 of 32 score 1 tumours had a poor response to CRT (grade 1), while 11 of 13 score 2 and both score 3 tumours had good CRT responses (grade 2 or 3) (*Table 2*).

Table 2 Correlation of sensitivity score with histological response of 66 oesophageal tumours to chemoradiation therapy

	Grade 1	Grade 2	Grade 3	Total
Score				
0	15	2	2	19 (29)
1	17	9	6	32 (48)
2	2	4	7	13 (20)
3	0	0	2	2 (3)
Total	34	15	17	66 (100)

Tumours were classified into four groups with scores of 0–3 by the addition of points: p53(+), metallothionein(+) and CDC25B(–)/(+), for which each point was 0; and p53(–), metallothionein(–) and CDC25B(+ +), for which each point was 1. Values in parentheses are percentages. $P < 0.001$ (Spearman's rank correlation test)

When clinical outcome was examined, patients with low biological CRT sensitivity scores (0 or 1) had a poorer prognosis than those with high scores (2 or 3) (5-year survival rate 34 *versus* 67 per cent respectively), although the difference was not statistically significant ($P = 0.12$) (*Fig. 3*).

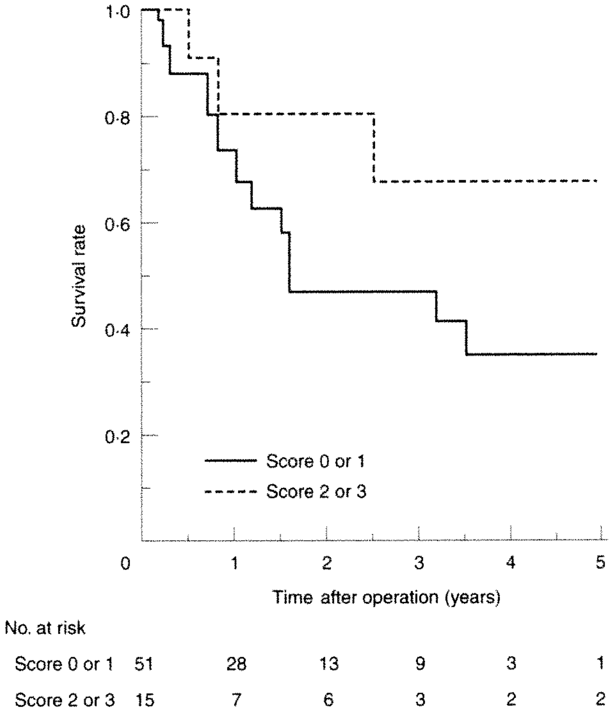


Fig. 3 Kaplan–Meier survival curves classified by tumour sensitivity score in 66 patients with oesophageal cancer. Patients with scores of 2 or 3 had an improved 5-year survival rate (67 per cent) compared with those with scores of 0 or 1 (34 per cent), but the difference was not statistically significant ($P = 0.12$, log rank test)

Discussion

Previous studies have implicated various molecules as candidates for biological markers for CRT response in human oesophageal cancer, including p53, CDC25B and MT[9,12]. In the present study, histological response to CRT was predicted more effectively by evaluating these three molecules simultaneously. This novel approach was successful because these three markers are involved via different pathways, and therefore served as independent factors for predicting the CRT response in multivariate analysis.

p53 is the best characterized and the most powerful marker, in addition to being the only established biological marker for response to CRT in both experimental[16] and clinical[17] studies. The G2–M checkpoint, which is preserved even in p53-impaired cells, is a major pathway of radiation-induced apoptosis[18]. DNA damage triggers the signal that activates ataxia telangiectasia mutated (ATM), ATM then phosphorylates and activates Chk2, and Chk2 phosphorylates and inactivates CDC25[19,20]. Inactivation of CDC25 results in suppression of CDC2/cyclin B1 activity and induction of G2–M arrest. G2–M arrest is indispensable for DNA repair following DNA damage, and abrogation of G2–M arrest facilitates the elimination of cells harbouring DNA damage via apoptosis during the G2–M phase. Among various molecules that regulate G2–M arrest, the overexpression of CDC25B has frequently been observed in human cancers[21,22] and was associated with response to CRT in a previous study[9]. Moreover, CDC25B overexpression in oesophageal cancer cells was found to abrogate G2–M arrest and induce apoptosis following irradiation[23]. Three members of the CDC25 gene family have been identified in human cells[24, 25], but only CDC25B is associated with CRT sensitivity. This is because CDC25A is not involved in the G2–M checkpoint but in the G1 checkpoint[26], and CDC25C is not overexpressed in cancer cells.

A controlled clinical study revealed that CRT, using cisplatin and 5-fluorouracil, was more effective than radiation alone for advanced oesophageal cancer[27]. MT is a metal-binding protein and detoxifies heavy metals[10]. In murine cell lines, transfection of the MT gene diminished cisplatin-induced apoptosis[28]. Expression of MT protein and messenger RNA was correlated with the cisplatin resistance in human lung cancer cell lines[29].

The present authors previously reported that the histological response to CRT was the only independent prognostic factor among clinicopathological parameters in patients with oesophageal cancer who had multimodal therapy[30]. In the present study, three biological markers were significantly related to the histological response to CRT, but none of them correlated individually with postoperative survival. CDC25B overexpression might have opposite effects on the prognosis: improving the response to CRT, but worsening prognosis as an oncogene facilitating G2–M progression. A controlled study comparing patients with or without CRT, subgrouped by CDC25B expression status, would determine the overall effect of CDC25B overexpression.

Patients with p53(–) tumours did not have a better postoperative prognosis following CRT, possibly because a grade 1 tumour response was seen as often in p53(–) tumours as a grade 3 response (*Table 1*). Generally, the p53(–) condition in immunohistochemistry is associated with the wild-type *p53* gene[31]. However, the grade 1 CRT response in this situation may imply complete loss of p53 protein due to stop codon mutation, homozygous deletion or acceleration of protein degradation[32]. Such *p53* null mutation was associated with a poor prognosis in lung cancer[33]. Both genomic and protein analysis would be necessary to reveal the true significance of p53 negativity in CRT resistance in human oesophageal cancer.

Prediction of the response to CRT by this approach could provide important information for clinical cancer treatment. It does rely on the reproducibility of immunohistochemical studies using biopsy specimens. Salvage operation after CRT might not be necessary following a grade 3 response, but may be indicated specifically after a grade 2 response. Unfortunately, there was little benefit from CRT with salvage operation in tumours with a grade 1 response[1]. Special treatments to increase CRT sensitivity might be required for non-responders. For example, restoring p53 function by gene therapy has been achieved in clinical trials[34], and a compound that stabilized the p53 protein has been reported recently[35]. Chemical compounds that regulate the G2–M checkpoint, such as 7-hydroxystaurosporine[36] or caffeine[37] are available clinically. MT expression was found to be reduced by proparglyglycine[38].

The findings of this study suggest that the choice of treatment for oesophageal cancer could be a complicated matter involving the evaluation of these biologically sensitive markers for response to CRT. In future, patients could have their individual therapy protocol based on the biological characteristics of their cancer cells in order to improve prognosis.

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Original article

Radio-guided sentinel node detection for gastric cancer

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Background: Radio-guided detection of sentinel nodes (SNs) has been used to predict regional metastases in patients with malignant melanoma and breast cancer. However, the validity of the SN hypothesis is still controversial for gastrointestinal cancers including gastric cancer. The aim of this study was to test the feasibility and accuracy of radio-guided mapping of SNs for gastric cancer.

Methods: Some 145 consecutive patients with gastric cancer diagnosed as T_1 or T_2 and evaluated clinically as N_0 were enrolled. Endoscopic injection of technetium-99m-radiolabelled tin colloid was performed before operation and radioactive SNs were identified with a γ probe. Standard radical gastrectomy with lymphadenectomy was performed in all patients and all resected nodes were evaluated by routine histopathological examination.

Results: Using radio-guided methods, SNs were detected in 138 (95·2 per cent) of 145 patients. The SN was positive in 22 of 24 patients with lymph node metastasis. The incidence of metastasis in the SNs (7·8 per cent) was significantly higher than that in the non-SNs (0·3 per cent) ($P < 0{\cdot}01$). The diagnostic accuracy according to SN status was 98·6 per cent (136 of 138).

Conclusion: Radio-guided SN mapping is an accurate diagnostic procedure for detecting lymph node metastasis in patients with early-stage gastric cancer.

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Introduction

For patients with gastric cancer, surgical resection with a lymph node dissection is considered a potentially curative treatment. Although gastrectomy with prophylactic lymph node dissection is the standard procedure in Japan and some other countries[1], the prognostic benefit of extended lymph node dissection (D_2) is still controversial[2]. Recently, Macdonald *et al.*[3] reported that only 10 per cent of 552 patients undergoing gastrectomy with curative intent actually had a formal D_2 dissection whereas most patients (54 per cent) had undergone a D_0 dissection (less than complete dissection of N_1 nodes). Completion of D_2 lymphadenectomy for gastric cancer is not always feasible and depends on the patient population and technical issues. Determination of the extent of lymph node dissection required in patients with gastric cancer on the basis of actual node involvement is important as less extensive dissection may reduce postoperative morbidity and mortality rates, especially in elderly patients. However, diagnostic imaging techniques including computed tomography (CT) and ultrasonography are still unsatisfactory and do not provide an accurate prediction of metastasis in the regional lymph nodes draining gastric cancer. Development of novel diagnostic methods for assessing lymph node metastatic status is crucial for accurate lymph node staging that would permit individualized minimally invasive surgery such as a laparoscopic wedge resection for patients with early gastric cancer.

The first possible sites of metastasis along the route of lymphatic drainage from a neoplastic primary lesion are known as sentinel nodes (SNs). These may be detected using injection of suitable dyes or radioactive tracers. In 1977, Cabanas[4] proposed that SNs in patients with penile cancer could be removed by limited surgery and the presence or absence of metastatic disease used to determine the need for more extensive lymphadenectomy. Morton and colleagues[5] first studied the concept of SNs in a feline model and later in a clinical study of patients with malignant melanoma[6]. They defined the SN as any node in the regional basin that had an afferent lymphatic connection from the primary tumour. This SN hypothesis has been tested by pilot studies in malignant melanoma and breast cancer[7–9]. Such studies have demonstrated that if the SNs are negative, it is unlikely that non-SNs contain micrometastatic disease. The accuracy of the status of the SN in predicting other node involvement in recent studies exceeds 95 per cent[10–12].

The feasibility of SN mapping in gastrointestinal cancers and its diagnostic reliability remains unclear. It is thought that nodal metastasis occurs randomly and that the second and third regional lymph node compartments may be involved in metastatic disease without involvement of the nodes closest to the primary gastrointestinal cancer lesion. However, Bilchik *et al.*[13] have recently proposed universal application of SN mapping to other solid tumours. If feasible, SN mapping would be of particular value in the management of early gastric cancer in which radical D_2 lymphadenectomy may be unnecessary.

Patients and methods

Patients consecutively treated at Keio University Hospital with clinically localized gastric cancer from January 1999 to May 2001 were offered enrolment in the study. Clinical diagnosis was made by examination of endoscopic biopsy specimens, and the absence of apparent lymph node involvement by diagnostic imaging was confirmed by preoperative CT and conventional ultrasonography. The depth of tumour invasion was evaluated on the basis of endoscopic findings and double contrast gastrography. One hundred and forty-five patients requiring curative gastrectomy with D_2 or modified D_2 lymphadenectomy (removal of all N_1 nodes and nodes along the left gastric artery and common hepatic artery) with a diagnosis of clinical T_1 or T_2, N_0 M_0 status were enrolled. The protocol was approved by an institutional review board and committee for radiation safety. All patients enrolled in this study gave written informed consent.

Technetium-99m-radiolabelled tin colloid solution in a volume of 2·0 ml was injected in four quadrants into the submucosal layer of the primary lesion using an endoscopic puncture needle. There were two groups which differed with respect to the timing of the tracer injection, either 2 h (n = 87) or 16 h (n = 58) before surgery owing to the operation schedule. To adjust for the activity at the time of surgery, the activity of injected technetium-99m-labelled tin colloid was 150 MBq at 16 h before surgery or 15 MBq at 2 h before surgery. Technetium-99m-labelled tin colloid solution was labelled by adding 1·5 ml tin (II) chloride solution (Nihon Medi-Physics, Nishinomiya, Japan) to 1·5 ml 99m-radiolabelled technetium pertechnetate solution containing 111 MBq of radioactivity.

A hand-held γ probe (Navigator; AutoSuture Japan, Tokyo, Japan) was used to locate the radioactive SNs. After identification of the SNs, standard total or distal gastrectomy with D_2 or modified D_2 lymphadenectomy was performed. After the resection, the absence of residual radioactivity in the abdomen was confirmed intraoperatively with a hand-held γ probe.

Table 1 Patient characteristics

Age (years)*	57(11)
Sex ratio (M : F)	107 : 38
Histological type†	
Well differentiated	27 (18·6)
Moderately differentiated	75 (51·7)
Poorly differentiated	26 (17·9)
Signet ring cell	17 (11·7)
Anatomical subsite†	
Upper third	22 (15·2)
Middle third	94 (64·8)
Lower third	29 (20·0)
Depth of invasion†	
T_1	127 (87·6)
T_2	18 (12·4)

*Values are mean(s.d.); †values in parentheses are percentages

The radioactivity of all the resected nodes was measured individually with a hand-held γ counter (window setting above 95 keV, measuring time 20 s) and a well-type scintillation counter (ARC-300; Aloka, Tokyo, Japan) (window setting 126–154 keV, measuring time 60 s). SNs were defined as nodes containing ten times more radioactivity than surrounding tissue. The number of hot nodes was compared between groups with different timing of the tracer injection.

A single haematoxylin and eosin-stained section of a formalin-fixed paraffin-embedded specimen of each lymph node harvested was examined by a pathologist who was blinded to the SN status. The pathological status of the SNs was compared with that of the remaining regional nodes (non-SNs). The successful identification rate of SNs, number, distribution and accuracy of SN assessment with respect to the positive or negative status of regional nodes were evaluated.

Statistical analysis

The incidence of metastases detected in SNs and non-SNs was compared by the χ^2 test.

Results

The clinicopathological characteristics of patients enrolled in the study are summarized in *Table 1*. There were no unexpected toxicities or complications related to administration of radioactive tracer.

Sentinel node identification

SNs were detectable as hotspots in 138 (95 per cent) of 145 patients with an intraoperative hand-held γ probe. In 138

Table 2 Distribution of the sentinel nodes in gastric cancer

Location of sentinel node*	No. of patients
Compartment 1 only	87 (63·0)
Compartment 1 and 2	43 (31·1)
Compartment 2 only	7 (5·1)
Compartment 1, 2 and 3	1 (0·7)
Compartment 3 only	0 (0)

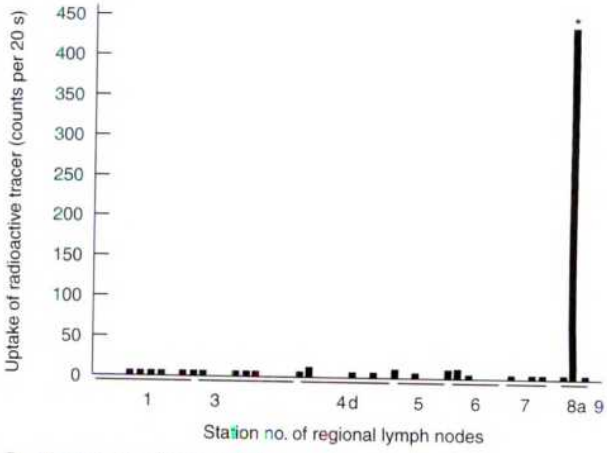
Values in parentheses are percentages. *Compartments of regional lymph nodes according to the Japanese Classification of Gastric Carcinoma[14]

patients radioactive tracer uptake was sufficient to identify the SNs. The counts measured by the hand-held γ counter and well-type scintillation counter were compatible. The number of lymph nodes removed by curative surgery with lymphadenectomy in this series was 45·5(19·2) (range 15–76) per patient. The number of SNs identified on the basis of radioactivity ratio criteria was 3·6(2·5) (range 1–8) per patient. The timing of tracer administration made no difference to the number of SNs identified (3·7 per patient with tracer injection 2 h before surgery ($n = 87$) and 3·4 per patient with tracer administered 16 h before surgery ($n = 58$)).

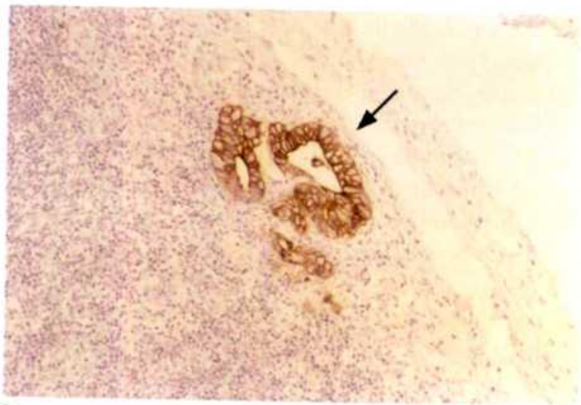
Sentinel node distribution

The location of identified SNs according to the Japanese Classification of Gastric Carcinoma[14] is indicated in *Table 2*. In seven patients (5·1 per cent), direct migration of radioactive tracer to the second compartment without incorporation in the first compartment was observed. In one of these seven patients, metastasis to a single SN in the second compartment was detected as shown in *Fig. 1*.

Metastases were detected by microscopic examination of a single haematoxylin and eosin-stained section of a formalin-fixed paraffin-embedded specimen in 24 patients. In 22 of these 24 patients, the SNs were positive for metastasis. The incidence of metastasis was significantly higher in SNs (7·8 per cent) than in non-SNs (0·3 per cent) ($P < 0{\cdot}01$). The overall diagnostic accuracy of assessment of regional lymph node status on the basis of SN status was 98·6 per cent (136 of 138).



a Radioactive tracer uptake



b Positive immunohistochemistry

Fig. 1 a Incorporation of radioactive tracer in resected lymph nodes measured by γ probe in a patient with superficial gastric cancer (T_1, sm_2). No uptake of radioactive tracer was observed in perigastric nodes (first compartment). The single lymph node along the common hepatic artery (8a; anterosuperior group, second compartment) showed high uptake of tracer (*) and was defined as the sentinel node in this patient. **b** The site of metastasis was limited to a very small cluster of cells (arrow) only in the sentinel node located in the second compartment (8a) and metastatic lesions were reconfirmed by immunohistochemistry using anticytokeratin antibody (original magnification × 50)

Discussion

In the present study the detection of SNs in patients with gastric cancer using technetium-99m-radiolabelled tin colloid was satisfactory. Endoscopic submucosal injection is considered a reasonable and feasible route of administration of radioactive tracer for lymphatic mapping of gastric cancers. The total number of SNs in each patient was relatively higher in this series of patients with gastric cancer than that previously reported in patients with breast cancer[10,11,15]. The number of SNs may be organ specific owing to the structure and anatomy of the regional lymphatic network. The number of SNs demonstrated in gastric cancer (3·6 per patient) is still within the practical range for intraoperative SN biopsy.

In the present study, the interval between injection of the tracer and resection varied (2 or 16 h) for practical reasons. However, there was no relationship between the interval of injection of radiocolloid to the SN harvest and the number of identified SNs. From these data, it appears that 2 h may be long enough for migration of the radiolabelled tin colloid; a long interval after injection (16 h) had no effect on the number of SNs identified. Tin colloid has a relatively large particle size in comparison to the sulphur nanocolloid commonly used and may be useful to avoid excessive diffusion. In the authors' experience, small particles such as activated carbon particles (CH40) are not suitable for detection of SNs because they migrate rapidly into too many regional lymph nodes (approximately 80 per cent). The radio-guided method allows quantitative detection of SNs because of stable incorporation of the radioactive tracer.

Joosten *et al.*[16] reported that lymphatic mapping and SN identification is not reliable in colorectal cancer using subserosal injection of blue dye. On the other hand, Saha *et al.*[17] were successful in identifying SNs in patients with colorectal cancer using the blue dye method. In gastrointestinal cancers, the lymphatic network may be more complex than that in breast cancer, and it may be difficult to visualize all the SNs distributed in unexpected areas by the vital blue dye method. In fact, the current data indicated a relatively high incidence (approximately one-third) of direct distribution of radioactive tracer in the second regional lymph node compartment in gastric cancer. This may be the major mechanism of so-called skip metastasis in gastric cancer. In these cases, SNs in the second compartment are considered functionally to be first compartment. *Fig. 1* illustrates a good example of the value of SN mapping in gastric cancer to detect what were previously labelled as skip metastases.

Intraoperative hand-held γ probing was useful in detecting SNs in functionally first compartments even though they were anatomically third-compartment nodes. Recently, a high diagnostic accuracy of lymph node metastasis by SN mapping in colorectal[18,19] and gastric[20] cancer using the blue dye method has been reported. In the present series, the high performance of radio-guided SN mapping in gastric cancer was demonstrated.

In the field of surgical treatment of gastric cancer, successful SN mapping has great potential to detect micrometastatic disease by focusing on SNs as selected targets for evaluation and for recognition of unusual lymph drainage. Furthermore, this technique has great potential application for development of novel minimally invasive surgical techniques. Curative laparoscopic surgery for mucosal gastric cancer has been reported[21]. Laparoscopic wedge resection with a safe surgical margin should be sufficient for curative treatment of early gastric cancer without lymph node metastasis. In fact, laparoscopic detection of SNs in gastric cancer using an abdominal γ probe (AutoSuture Japan, Tokyo, Japan) was feasible in a preliminary study[22]. Several issues, such as the sensitivity of intraoperative pathological examination and the technique of laparoscopic SN detection, remain to be resolved before wide clinical application of this procedure. However, from this initial experience, it is reasonable to conclude that radio-guided SN detection has potential value in the staging and treatment of patients with early gastric cancer.

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Original article

Extended preservation of non-heart-beating donor livers with normothermic machine perfusion

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Background Non-heart-beating donor (NHBD) livers represent an important organ pool, but are seldom utilized clinically and require rapid retrieval and implantation. Experimental work with oxygenated perfusion during preservation has shown promising results by recovering function in these livers. This study compared sanguinous perfusion with cold storage for extended preservation of the NHBD liver in a porcine model.

Methods Porcine livers were subjected to 60 min of *in vivo* total warm ischaemia before flushing, after which they were preserved by one of two methods: group 1 ($n = 4$), University of Wisconsin (UW) solution by standard cold storage for 24 h; group 2 ($n = 4$), oxygenated autologous blood perfusion on an extracorporeal circuit for 24 h. All livers were subsequently tested on the circuit during a 24-h reperfusion phase.

Results Livers in group 1 showed no evidence of viability during the reperfusion phase with no bile production or glucose utilization; they also displayed massive necrosis. Livers in group 2 demonstrated recovery of function by synthetic function, substrate utilization and perfusion haemodynamics; these livers displayed less cellular injury by hepatocellular enzymes. All differences in parameters between the two groups were statistically significant ($P < 0{\cdot}05$). These findings were supported by histological examination.

Conclusion Warm ischaemia for 1 h and simple cold storage (UW solution) for 24 h renders the liver non-viable. Oxygenated, sanguinous perfusion as a method of preservation recovers liver function to a viable level after 24 h of preservation.

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Introduction

Non-heart-beating donor (NHBD) livers represent a potential pool of organs that could significantly reduce the large discrepancy between supply and demand for liver transplantation[1]. The current method of preserving livers by simple cold storage subjects the organ to a further period of ischaemia, increasing the level of injury, and usually leaving the organ unfit for transplantation[2]. The cold-stored NHBD organ undergoes injury at four consecutive stages: (1) prior to retrieval (warm ischaemia), (2) during cold storage ischaemia, (3) during ischaemic rewarming (warm ischaemia) and (4) at reperfusion. Consequently, clinical experience with the NHBD liver has been limited[3–5]. When cardiac arrest occurs outside the operating room, there is a high rate of primary non-function[4]. It is for these reasons that the NHBD liver is rarely used; however, in view of the excess of demand over supply in liver transplantation, and the lack of any alternative means of providing long-term support to patients with liver failure, it is important to develop methods that make use of NHBD livers.

The use of NHBD donor livers that have experienced substantial injury from warm ischaemia during the period of arrest would require the capability of ameliorating the ischaemic cellular changes either before or during the preservation period. It was hypothesized that perfusing the liver with an oxygenated perfusate supplemented with nutritional support at body temperature during the preservation period might be a way of accomplishing this goal. By these means, depleted cellular substrates could be restored during preservation, allowing the organ to tolerate the warm ischaemic period of surgical anastomosis and reperfusion.

This study investigated the use of oxygenated, normothermic perfusion of NHBD livers during the preservation period as an alternative to cold storage. Preservation by normothermic blood perfusion was compared directly with simple cold storage in the porcine model after 60 min of warm ischaemia.

Methods

Operation

White Landrace pigs (40 kg) were premedicated intramuscularly with ketamine (Ketaset®; IDIS, Surbiton, UK) 10 mg/kg and midazolam (Hypnovel®; Roche, Welwyn Garden City, UK) 1 mg/kg. General anaesthesia was induced with propofol (Diprivan®; AstraZeneca, King's Langley, UK) 0·5 mg/kg given intravenously, and maintained by an intravenous infusion of propofol (1 mg per kg per h) together with halothane inhalation via an extended endotracheal tube.

Upon laparotomy the porta hepatis was dissected to delineate the anatomy. The common bile duct was ligated and divided. The infrahepatic vena cava, portal vein and arterial anatomy were each identified. After 20 000 units of standard human heparin (Multiparin®; CP Pharmaceuticals, Wrexham, UK) had been injected directly into the aorta and allowed to circulate, a 10-Fr cannula (Medtronic, Watford, UK) was placed into the abdominal aorta and 4 units blood were taken. Cardiac arrest was then induced by intracardiac injection with 10 ml potassium chloride. A period of circulatory arrest of exactly 60 min was observed in every case before flushing the liver with 3 litres cold Eurocollins solution (Soltran®; Baxter, Compton, UK). The livers were then assigned to a preservation period of either simple cold storage in University of Wisconsin (UW) solution (Viaspan®; Du Pont, Stevenage, UK) or machine perfusion according to the experimental design outlined below.

Experimental design (*Fig. 1*)

The livers were the preserved for 24 h in one of two groups: group 1 ($n = 4$) – flushed and preserved in cold storage for 24 h in UW solution; group 2 ($n = 4$) – placed on an isolated perfusion circuit with whole blood at 38°C.

Reperfusion period

All livers were tested for function after the preservation period by using an extracorporeal circuit to perfuse the livers with whole blood at body temperature as a surrogate for transplantation. To replicate surgical anastomosis, both groups were flushed after preservation with 2 litres non-oxygenated cold Eurocollins solution and left for 45 min before being flushed with a warm plasma substitute (Gelofusine®; B. Braun, Sheffield, UK) and reperfused on

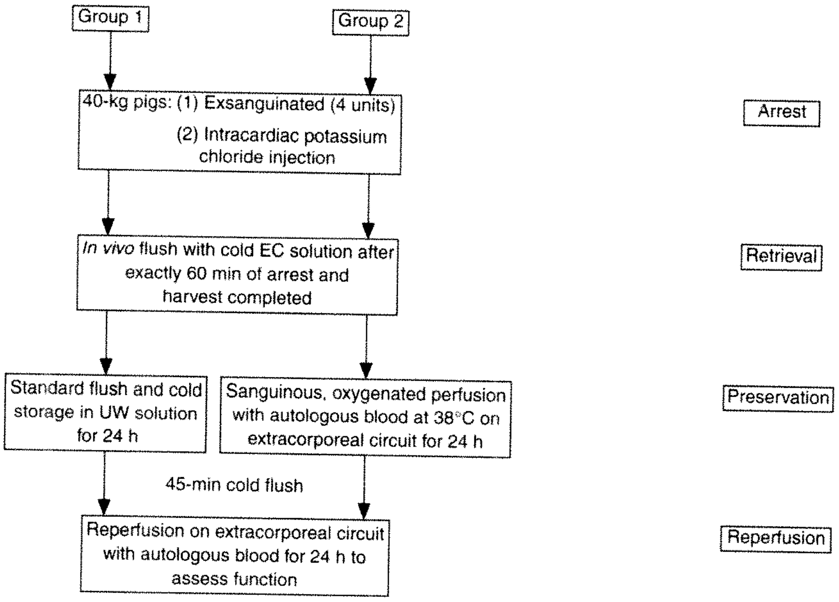

Group 1
Group 2
40-kg pigs: (1) Exsanguinated (4 units)
(2) Intracardiac potassium chloride injection
Arrest
In vivo flush with cold EC solution after exactly 60 min of arrest and harvest completed
Retrieval
Standard flush and cold storage in UW solution for 24 h
Sanguinous, oxygenated perfusion with autologous blood at 38°C on extracorporeal circuit for 24 h
Preservation
45-min cold flush
Reperfusion on extracorporeal circuit with autologous blood for 24 h to assess function
Reperfusion

Fig. 1 Experimental design. EC, Eurocollins; UW, University of Wisconsin

the extracorporeal circuit. The blood used to perfuse livers in group 2 was changed between the preservation and reperfusion phases so that a fair comparison of physiological parameters could be made.

Assessment of function

Arterial blood gases, venous blood gases, blood glucose, haemodynamics and bile production were also documented every hour of the reperfusion phase. Perfusate was sampled every hour for the first 8 h of the reperfusion period and every 4 h thereafter for conventional liver function testing and estimation of urea and electrolyte levels.

Full blood count and factor V samples were taken every 2 h for the first 8 h, and then every 4 h. Galactose clearance was measured at 12 and 24 h of reperfusion. At the end of each perfusion, the liver was sectioned and multiple random samples were assessed histologically for evaluation of reperfusion injury.

Perfusion circuit

The circuit consisted of an oxygenator (1500 ECMO Oxygenator; Medtronic), a heat exchanger (Ecmotherm II HE; Medtronic), a centrifugal pump (BP50 Centrifugal Pump; Medtronic), an 800-ml venous reservoir bag (Medtronic) and polyvinylchloride tubing (Medtronic) with an internal diameter of 1/4 and 3/16 inches (*Fig. 2*). During priming, the perfusion circuit was supplemented with 9·2 mmol calcium chloride, 20 mmol sodium bicar-

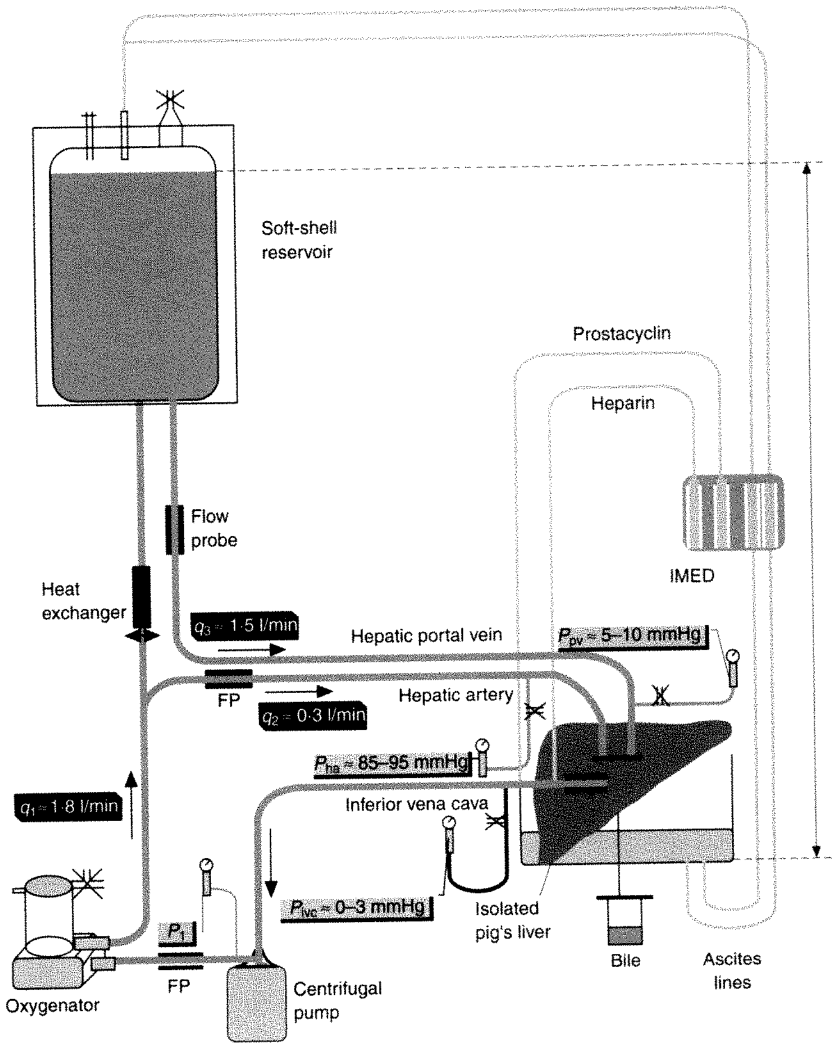


Fig. 2 Extracorporeal perfusion circuit. FP, flow probe; IMED, infusion pump; *P*, pressure, *q*, flow; ivc, inferior vena cava; ha, hepatic artery; pv, portal vein

bonate and 7000 units heparin in all cases. Temperature was maintained at 38°C. The circuit was supplemented with a continuous infusion of prostacyclin (Flolan®; Glaxo Wellcome, Uxbridge, UK) at 4 ml/h, 2 per cent taurocholic acid (Sigma, Watford, UK) at 7 ml/h and nutrition (Nutriflex®; B. Braun) at 17 ml/h. Nutrition consisted of 96 g amino acids and 300 g anhydrous glucose in 2 litres of solution, while fats were excluded. Regular human insulin (Humulin®; CP Pharmaceuticals), 100 units, was added to each bag. Heparin, 3000 units, was added every 4 h.

While attempting to keeping the extracorporeal circuit user-friendly, it is imperative that it simulates the *in vivo* physiological environment as closely as possible. Thus it was made up of two circuits in parallel, the arterial arm being fed directly from the centrifugal pump (high pressure) and the portal arm being fed passively from a venous reservoir by gravity (low pressure). Efferent was retrieved via the suprahepatic caval cannula and blood returned to the pump, after which the blood was oxygenated and warmed. During preservation, physiological flows and pressures were maintained in the hepatic artery, portal vein and vena cava by controlling pump head speed and adjustment of gate clamp resistance. At the initiation of the reperfusion phase, the pump was maintained at a set rate (2600 cycles per minute) so that the flows and pressures within the vessels could be used as an indication of viability.

Statistical analysis

Statistical analysis was performed using Welch's t test (assumption of unequal variances), with each timepoint reflecting the mean of four perfusions. $P \leq 0.05$ was considered significant. The comparisons were made between the two groups for each individual parameter at each equal timepoint in perfusion.

Results

Upon reperfusion, none of the cold-stored livers of group 1 demonstrated any sign of viability throughout the reperfusion phase. The machine-perfused livers in group 2 functioned well throughout the reperfusion phase. The comparative data are outlined below.

Hepatocellular damage

Mean levels of alanine aminotransferase (ALAT) rose sharply upon reperfusion in group 1 livers (*Fig. 3*) compared with levels seen in group 2. This was statistically significant by 1 h of reperfusion ($P = 0.016$) and at all timepoints thereafter. The rise in ALAT concentration continued throughout reperfusion of the livers in group 1, with relatively little increase seen in group 2 during the same time period. Mimicking the ALAT curves, aspartate aminotransferase (ASAT) levels in group 1 livers rose rapidly to 3810 units/l after 1 h of reperfusion, whereas the mean level of ASAT in group 2 was only 259 units/l after 1 h of reperfusion ($P = 0.036$). The differences between the two groups were significant at all timepoints. The mean level of lactate dehydrogenase rose to 2985 units/l in group 1 livers by 1 h of reperfusion, compared with 1158 units/l in group 2. The differences between the two groups continued to grow throughout reperfusion and were statistically significant from 2 h onwards.

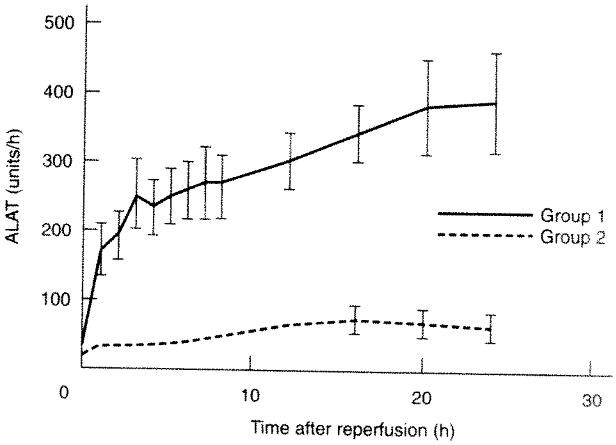


Fig. 3 Demonstration of massive hepatocellular lysis in group 1 livers with a progressive rise in alanine aminotransferase (ALAT) levels. Values are the mean(s.e.m.) of four experiments. $P \leq 0.05$ for all timepoints (Welch's t test)

Synthetic function

The cold-stored livers of group 1 produced no bile after reperfusion in any of the four perfusions. In addition, these livers displayed haemorrhagic output from the biliary system. The perfused livers of group 2 recovered bile function of up to 10 ml/h. The differences were statistically significant at all timepoints (*Fig. 4*).

Factor V levels fell in group 1 to 12 units/dl at 24 h, while livers in group 2 maintained synthetic function throughout reperfusion, with a level of 263 units/dl at 24 h. The differences were statistically significant from 4 h onwards.

Metabolic function

Livers in group 1 showed no evidence of glucose consumption. Perfusate glucose levels remained above the upper limit of detection throughout reperfusion. In contrast,

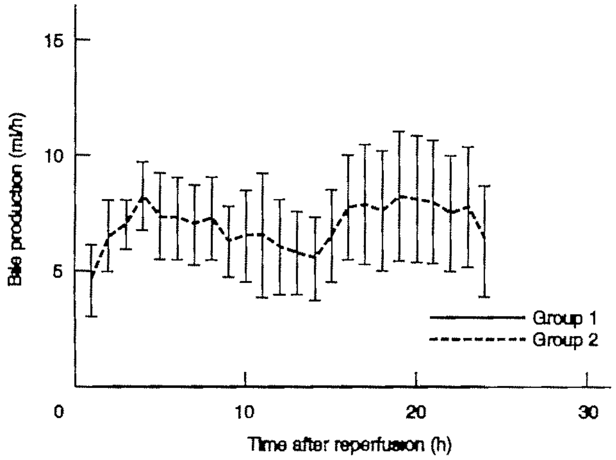


Fig. 4 Group 2 livers produced bile throughout the reperfusion phase, whereas group 1 livers made no bile. Values are the mean(s.e.m.) of four experiments. $P \leq 0{\cdot}05$ for all timepoints (Welch's *t* test)

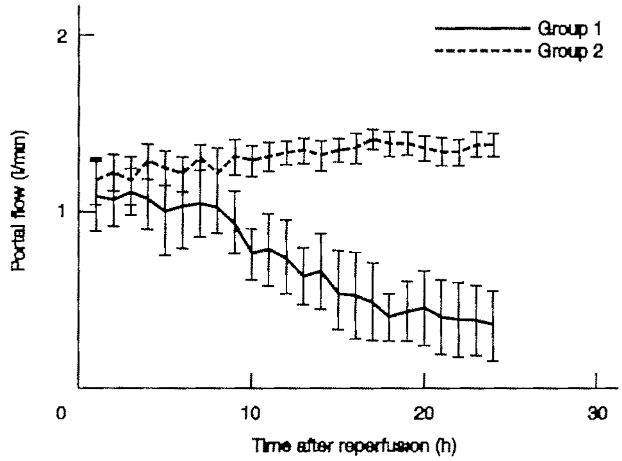


Fig. 6 Portal flow deteriorates throughout reperfusion in group 1 livers, presumably due to progressive destruction of sinusoidal and tissue architecture. Values are the mean(s.e.m.) of four experiments. $P \leq 0{\cdot}05$ for all timepoints after 10 h (Welch's *t* test)

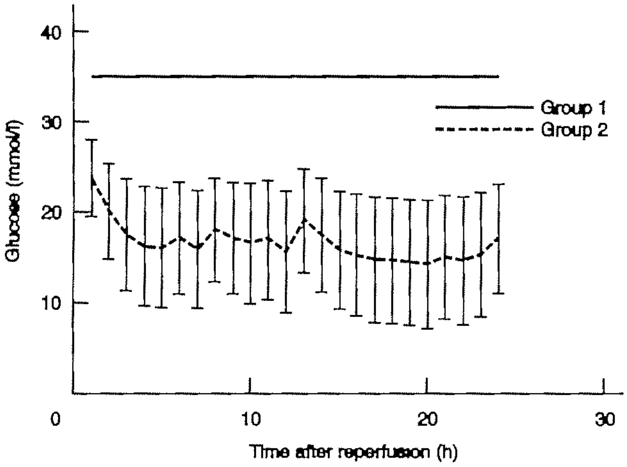


Fig. 5 Metabolic activity is still present after 24 h of machine preservation as demonstrated by the glucose utilization curve for group 2 livers. Values are the mean(s.e.m.) of four experiments. $P \leq 0{\cdot}05$ for all timepoints (Welch's *t* test)

group 2 livers demonstrated glucose utilization throughout reperfusion (*Fig. 5*).

Haemodynamics

The mean total hepatic blood flow in group 1 decreased throughout the reperfusion phase to 0·44 l/min at 24 h, compared with 1·40 l/min at 24 h in group 2 ($P = 0{\cdot}005$).

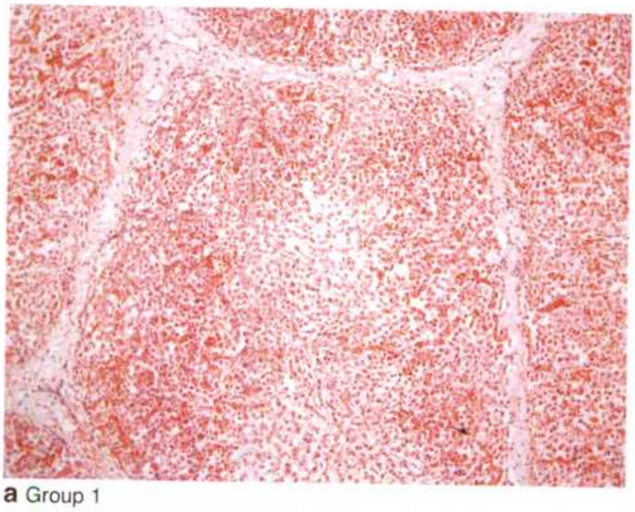
Both portal and arterial flow followed similar curves, with diminishing values seen in group 1. The portal flow, similar to total flow, was significantly greater in group 2 livers, with a widening difference throughout reperfusion (*Fig. 6*).

Haemolysis

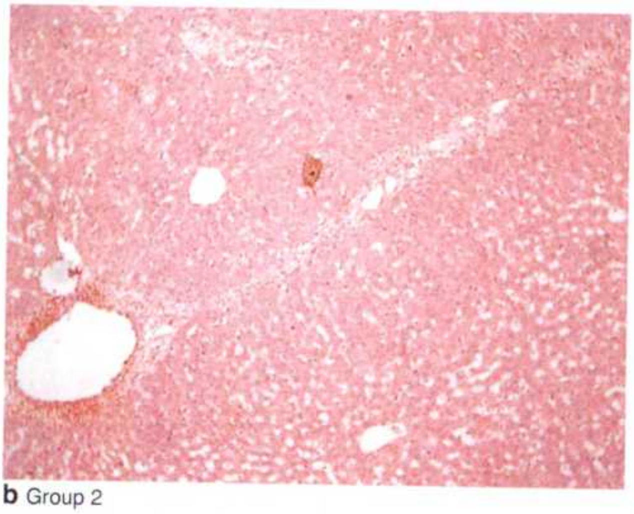
Despite both groups having no significant difference in haemoglobin concentration at the commencement of reperfusion (mean(s.d.) 7·6(1·3) g/dl in group 1 *versus* 6·8(2·2) g/dl in group 2), the perfusate in group 1 demonstrated massive haemolysis immediately, such that the blood gas machine could no longer detect the sample shortly after reperfusion. A measurable level of haemoglobin was present in the 2-h sample in only one of the experiments from this group (mean 0·98 g/dl at 2 h). By contrast, livers in group 2 maintained measurable haemoglobin levels throughout reperfusion. The mean haemoglobin at 2 h was 5·55 g/dl ($P = 0{\cdot}02$).

Histological findings

The group 2 livers showed diffuse, mild vacuolization and sinusoidal dilatation with minimal to no necrosis and preserved tissue structure. Livers in group 1 displayed diffuse hepatocellular necrosis (more than 66 per cent per high-power field), sinusoidal haemorrhage and architectural destruction (*Fig. 7*).



a Group 1



b Group 2

Fig. 7 **a** Gross hepatocellular necrosis, sinusoidal haemorrhage and architectural destruction diffusely present. **b** Mild vacuolization and sinusoidal dilatation with viable hepatocytes and preserved tissue structure

Discussion

This experiment was based on data that oxygenated perfusion of NHBD livers will promote cellular recovery from warm ischaemic injury. Without perfusion, cold storage exacerbates the injury by subjecting the organ to additional (cold) ischaemia; then, during surgical anastomosis in the recipient, another period of warm ischaemia. These consecutive ischaemic periods facilitate the progression of cellular injury to a state that is not compatible with recovery upon reperfusion. It is therefore essential to restore cellular energy levels between retrieval and reimplantation for NHBD livers.

Previous studies have shown that perfusion prior to harvesting of the NHBD liver can restore cellular energy levels before cold storage. Total body reperfusion with cardiopulmonary bypass (CPB) using autologous blood after 30 min without ventilation was shown to revive total adenosine 5′-triphosphate (ATP) content in porcine livers and kidneys[6]. Conditioning the liver with 30 min of normothermic isolated perfusion *in vivo*, using whole blood, has been shown to increase mitochondrial ATP content after 10 min of circulatory arrest in the porcine model[7]. CPB for only 10 min after 30 min of arrest, at 37°C, provided functional recovery of the heart, liver and kidney in the canine model[8]. In the porcine transplant model, CPB for systemic perfusion with cold, oxygenated Eurocollins solution restored tissue energy charge after 10 min of arrest[9]. These livers were then cold-stored for 5 h and transplanted with a survival rate of 100 per cent in the perfused group, while there were no recipient survivors in the control group (no perfusion). The largest study utilizing preharvest perfusion to salvage NHBD livers in the transplant model comes from Barcelona[10]. In this study, pigs were assigned progressive circulatory arrest time, followed by CPB to perfuse the livers *in vivo* for 30 min prior to cold storage and transplantation. The control group did not undergo bypass before cold storage. The transplant survival rate was related significantly to warm ischaemia time. However, even after 40 min of arrest, 50 per cent survived in the perfused group, whereas there were no survivors in the control group after only 20 min of arrest. This work showed that bypass did regenerate tissue energy charge, and that maintenance of these energy stores after arrest correlated with post-transplant survival[11,12].

Cellular substrates can also be restored after the NHBD liver has been harvested. Sanguinous perfusion of isolated porcine livers at body temperature for 3 h has been shown to resuscitate basic liver function on a perfusion circuit after 75 min of arrest[13]. In this study, perfusion was initiated immediately after harvest, representing the final phase of the experiment. This shows that sanguinous perfusion upon retrieval does restore liver integrity after a period of warm ischaemia. The present study confirmed these findings with the excellent function observed in group 2 livers during the preservation period.

Delivery of oxygen during the preservation period has previously been shown to be successful in the NHBD model by various methods. Persufflation of oxygen during cold storage in UW solution resulted in 100 per cent recipient survival in an experimental group of pigs after 45 min of arrest, while all the controls without persufflation died[14]. In a separate study, machine perfusion of the pig liver through the hepatic artery with acellular perfusate (UW-gluconate) resulted in a transplant survival rate of 80 per cent after

90 min of hypotension and a brief period of arrest, while the control group (cold storage) displayed a 25 per cent survival rate[15].

Schon *et al.*[16] subjected porcine livers to 1 h of warm ischaemia before harvesting, but by preserving them for 4 h with normothermic extracorporeal perfusion using blood as the perfusate they were able to display excellent post-transplant function in a group of six pigs. Also in this study, a group of six pigs with the same warm ischaemia time showed no survivors after preservation in cold UW solution for 4 h. This work clearly delineates the benefit of sanguinous perfusion during preservation, but it should be noted that the preservation period was only 4 h, which is of marginal clinical applicability. Also, their perfusion circuit included diaphragmatic replication and dialysis, based on previous work[17–19]. In the present study excellent results were achieved after 24 h of perfusion without these additions. Schon's group did not provide nutrients, bile salts or prostacyclin for their perfused livers.

In the present study normothermic perfusion was used for an extended preservation period of 24 h. To simulate the transplant model as closely as possible, the livers in group 1 were taken off the circuit after 24 h of machine preservation, and a 45-min period of ischaemia was initiated by flushing with cold Eurocollins solution followed by warm colloid. This subjected the livers to ischaemic periods of cooling and rewarming before reperfusion.

The results of this experiment show, as expected, that 1 h of warm ischaemia followed by 24 h of cold storage in UW solution results in a completely non-viable organ. However, 24 h of normothermic perfusion preservation produces a functioning liver, despite the same ischaemic injury. The perfused livers in group 2 made bile, utilized glucose, maintained stable haemodynamics and displayed histological features of viability, confirming the hypothesis that perfusion during the preservation period restricts the level of ischaemic injury in the NHBD liver. These results provide a powerful argument for the value of oxygenated perfusion during preservation as the length of both the arrest time and the preservation time were longer than those in other studies. A preservation period of 24 h is much longer than the pig liver will normally tolerate, yet the livers in group 2 demonstrated good preservation even when this was compounded by 60 min of arrest. Furthermore, perfusing NHBD livers throughout the preservation period not only replenished cellular substrate, ameliorating the ischaemic injury, but provided a clear assessment of liver function. This technique, in combination with the use of a variety of viability markers, could permit the use of severely injured organs with reassurance of function.

It is concluded that sanguinous perfusion as a method of preservation is potentially a technically feasible means of providing viable organs after significant warm ischaemic injury and an extended period of preservation. The next step in validating this technique of perfusion preservation to salvage NHBD organs will require demonstration of function and recovery after transplantation.

Acknowledgements

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Original article

Experimental study of peritoneal blood flow and insufflation pressure during laparoscopy

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Background: Port-site metastases after laparoscopic surgery may occur with greater frequency than would be expected following open resection of intra-abdominal malignancies, but the causal mechanism for this is incompletely understood. The possibility that insufflation may increase peritoneal blood flow producing a wound environment conducive to the formation of metastases was investigated.

Methods: The effects of insufflation gas type and pressure were studied in 30-kg female pigs. Pigs were divided into five groups, which were subjected to insufflation at 12 mmHg pressure with helium, insufflation at 12, 8 or 4 mmHg pressure with carbon dioxide, or laparotomy. A microsphere technique utilizing two distinct radiotracers, ^{99m}Tc-labelled macroaggregated albumin (MAA) and ^{51}Cr-labelled MAA, was used to study blood flow to the peritoneum, liver and kidneys.

Results: Insufflation with carbon dioxide or helium gases had no effect on renal ($P < 0{\cdot}09$) or hepatic blood flow ($P = 0{\cdot}54$). However, insufflation significantly increased peritoneal blood flow when carbon dioxide ($P < 0{\cdot}05$), but not when helium ($P = 0{\cdot}99$), was used as the insufflating gas.

Conclusion: These data suggest that blood flow within the peritoneum is influenced by insufflation with carbon dioxide. It is conceivable that such hyperaemia could increase the propensity for implanted tumour cells to metastasize in these sites following laparoscopy.

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Introduction

While a majority of surgeons might be expected to use a laparoscopic approach for procedures such as cholecystectomy and fundoplication, fewer use laparoscopic techniques for appendicectomy and hernia repair, and fewer still for colectomy. Laparoscopic colectomy has failed to gain widespread adoption for several reasons. Enthusiasm was damped by initial studies in which a relatively high number of patients developed wound (port site) metastases[1], and there is continuing debate both over the oncological safety of this procedure and the true incidence of wound metastasis compared with that in open surgery. Additionally, laparoscopic colectomy can be technically complex, with a long learning curve and a high conversion rate[2,3]. Despite this, there are proven benefits for patients following laparoscopic colectomy, including reduced hospital stay, less postoperative pain and improved cosmesis[4].

Previous research has demonstrated that the direct spread of tumour cells to trocar sites is a feasible causal explanation for at least some trocar-site metastases[5]. However, as direct spread is also likely to occur to open wounds during laparotomy, it is difficult to explain why trocar-site metastases might occur with greater frequency than wound metastases.

The present study was designed to test two hypotheses: first, that insufflation with carbon dioxide produces local vasodilatation in the peritoneum and thus may improve the 'soil' for the metastatic 'seeds' to implant in and, second, that helium does not alter peritoneal blood flow as this gas is biologically inert. Furthermore, since abdominal insufflation can produce oliguria[6,7] and occasionally even acute renal failure[8], the effect of insufflation with carbon dioxide at varying pressures and of an alternative gas (helium) on blood flow in other intra-abdominal tissues was also investigated.

Materials and methods

Preparation of radiopharmaceuticals

^{99m}Tc-radiolabelled macroaggregated albumin (MAA) was prepared using an MAA cold kit (TechneScan®; Mallinckrodt Medical, St Louis, Missouri, USA) to which was added 60–120 MBq ^{99m}Tc-radiolabelled sodium pertechnetate in 5 ml saline. A mean(s.e.m.) dose of 16·3(2·5) MBq ^{99m}Tc-labelled MAA was administered. ^{51}Cr-labelled MAA was prepared as reported elsewhere[9]. Briefly, 3·25 mg sodium dithionite in 1 ml water for injection was added to an MAA cold kit, followed by 4 MBq sodium [^{51}Cr]chromate in 4 ml saline (Australian Radioisotopes, Sydney, New South Wales, Australia). After 10 min at room temperature, the kit was centrifuged at 700–800*g* for 5 min. The supernatant was discarded and the pellet was washed twice before being resuspended in 4 ml 0·9 per cent saline. A mean(s.e.m.) dose of 1·6(0·6) MBq ^{51}Cr-labelled MAA was administered. The ^{51}Cr-labelled MAA and ^{99m}Tc-labelled MAA doses each had an equal number of particles (2·9(1·4) 10^6).

Radiochemical purity (RCP) was determined using the membrane filtration method[10]. Only radioactive kits with more than 95 per cent RCP were used in these experiments.

Operative protocol

Experiments performed with pigs complied with the code of practice for the care and use of animals in research in Australia, National Health and Medical Research Council, 1997. The protocol was approved by the animal ethics committees of the Institute of Medical and Veterinary Science, University of Adelaide and Queen Elizabeth Hospital.

Twenty-five female domestic 30-kg pigs were used. They were were allowed clear fluids only for 12 h before operation. Pigs were divided into five groups: group 1, insufflation at a pressure of 12 mmHg with helium; group 2, insufflation at a pressure of 12 mmHg with carbon dioxide; group 3, insufflation at a pressure of 8 mmHg with carbon dioxide; group 4, insufflation at a pressure of 4 mmHg with carbon dioxide; and group 5, laparotomy.

The pigs were premedicated with intramuscular ketamine 300 mg (Troy Laboratories, Smithfield, New South Wales, Australia) and anaesthesia was maintained by halothane (induction 3 per cent, maintenance 1·5 per cent) via a cuffed endotracheal tube. Each pig was secured by adhesive tape (Millipore, Bedford, Massachusetts, USA) to an operating table in a supine position. The left femoral artery was then cannulated and a radio-opaque catheter (6-Fr twin-lumen central venous line; Arrow, Reading, Pennsylvania, USA) was advanced into the descending aorta; the latter was screened fluoroscopically to confirm its position before it was secured with a 0 silk ligature (Ethicon, North Ryde, New South Wales, Australia).

The right carotid artery was exposed similarly and bathed in 2 ml 1 per cent lignocaine solution (Astra Pharmaceuticals, North Ryde, New South Wales, Australia) before being cannulated with a catheter (8-Fr coronary catheter (Cordis™; Johnson and Johnson, Ascot, UK)). This catheter was advanced into the left ventricle; its position was confirmed fluoroscopically and checked by injecting 10 ml contrast agent (Urovision®; Schering, Tempe, New South Wales, Australia), after which it was secured with a silk ligature.

A dose of 20 MBq ^{99m}Tc-labelled MAA in 1 ml was injected into the left ventricle followed by a flush with 5 ml 0·9 per cent saline. Ten seconds before this injection, a reference blood sample was withdrawn from the descending aorta over 2 min at 10 ml/min by a Harvard infusion/withdrawal pump (Stansen Scientific, Collingwood, Victoria, Australia). In the laparotomy group, a 15-cm midline laparotomy incision was made, which was then covered by a moist saline-soaked pack. In the insufflation groups, three 11-mm laparoscopic trocars (Endopath®; Johnson and Johnson Medical, North Ryde, New South Wales, Australia) were inserted at the umbilicus, right iliac fossa and left hypochondrium. The abdomen was insufflated to 4, 8 or 12 mmHg pressure with carbon dioxide gas or to 12 mmHg with helium. After the laparotomy or insufflation had been established for 30 min, 2 MBq ^{51}Cr-labelled MAA in 1 ml was injected into the left ventricle, followed by a 5-ml saline flush. A further blood sample was obtained from the descending aorta.

Pigs were then killed by intravenous injection of 20 ml Lethobarb® (pentobarbitone 325 mg/ml; Virbac Australia, Peakhurst, New South Wales, Australia). If not already done, a laparotomy was performed and both kidneys were removed. Any residual blood was allowed to drain from these organs before they were placed in plastic specimen bags, sealed and weighed. The peritoneum was stripped from the anterior abdominal wall on both sides until it became attached posteriorly, and then sealed in a plastic bag and weighed.

Data acquisition

The radioactivity of each organ and both reference blood samples were all counted at 24 h for ^{99m}Tc and at 6 days for ^{51}Cr deposition. A previous validation confirmed that at a ^{99m}Tc : ^{51}Cr dose ratio of 20 : 2, ^{51}Cr γ-energy emissions were sufficiently low as to have no effect on the ^{99m}Tc counts at 24 h after injection for each specimen, and that no

Table 1 Renal blood flow changes measured in anaesthetized animals before and after laparoscopy or laparotomy

	Blood flow (ml per min per 100 g)		
Group	Before	After	P*
12 mmHg helium	86·9(17·4)	88·2(21·6)	0·891
12 mmHg carbon dioxide	157·3(57·8)	143·4(69·5)	0·359
8 mmHg carbon dioxide	206·3(44·3)	106·0(12·7)	0·001
4 mmHg carbon dioxide	174·4(31·4)	144·8(21·7)	0·398
Laparotomy	96·8(21·1)	69·7(10·5)	0·005

Values are mean(s.e.m.). *t test

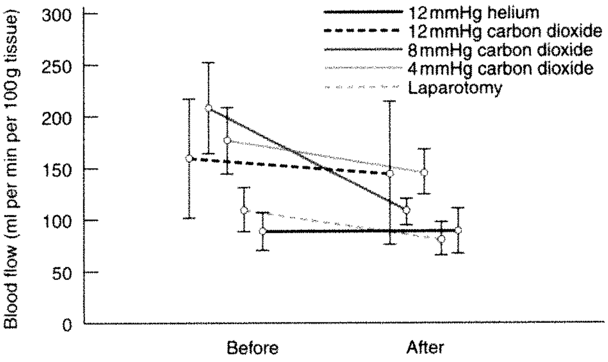

Fig. 1 Mean(s.e.m.) renal blood flow measured in anaesthetized animals before and after laparoscopy or laparotomy

interference from residual ^{99m}Tc energy affected ^{51}Cr counts of the same specimens after 6 days.

The specimens were counted in triplicate in a large-volume counter (Biosentry; AEI-EKCO, Australia) linked to a multichannel analyser (Model 3100; Canberra Industries, Meriden, Connecticut, USA), over a 130–150-KeV ^{99m}Tc window and a 250–400-KeV ^{51}Cr window. Dissected organs and blood samples were stored in a refrigerator (4°C) during the counting period. All counts were adjusted for background activity and decay.

In calculating organ blood flows, the number of counts within each reference blood sample (r_{chromium}, $r_{\text{technetium}}$) was divided by the volume of blood (V) and 2-min collection period according to the formula $c = r/2V$, to give counts per millilitre per minute of blood (c_{chromium}, $c_{\text{technetium}}$). Blood flow (b_{renal}, $b_{\text{peritoneum}}$) was then determined by dividing the isotope-specific counts within each organ (o_{renal}, $o_{\text{peritoneum}}$ etc.) with corresponding values from the reference blood samples above according to the formula $b = o/c$.

Statistical analysis

Values are expressed as mean(s.e.m.). Differences between baseline and treatment values were analysed by paired *t* test to determine the effect of each insufflation pressure. SPSS (version 10.0) (SPSS, Chicago, Illinois, USA) was used to analyse data using paired and independent-sample *t* tests. Hierarchical linear modelling (HLM/2L, version 5.0; Scientific Software International, Chicago, Illinois, USA) was used to determine whether insufflation pressure or gas type (carbon dioxide *versus* helium) influenced organ blood flow, the percentage variance and variance type of the composite data. $P < 0.05$ was considered statistically significant for univariate and multivariate analyses.

Results

Of the total group of 25 pigs, three died under anaesthesia from malignant hyperthermia before completion of the experimental protocol. Autopsies were performed on all these animals and no other abnormalities were detected.

Blood flow results were available for five pigs that had insufflation with helium at a pressure of 12 mmHg (group 1), four pigs that had insufflation with carbon dioxide at a pressure of 12 mmHg (group 2), five pigs that had insufflation with carbon dioxide at a pressure of 8 mmHg (group 3), three pigs that had insufflation with carbon dioxide at a pressure of 4 mmHg (group 4) and five pigs that had a laparotomy (group 5).

Renal blood flow

Mean renal blood flow was 14·0(18·0) ml per min per 100 g renal tissue before the creation of pneumoperitoneum or a laparotomy wound. Renal blood flow was unchanged in animals when a pneumoperitoneum was created with carbon dioxide at 12 and 4 mmHg, but it was significantly decreased at 8 mmHg. Insufflation with helium at 12 mmHg had no significant effect on renal blood flow; however, creation of a laparotomy wound significantly decreased renal blood flow (*Table 1* and *Fig. 1*). There was a wide variation in renal blood flow between animals, although concordance between left and right renal blood flows was good ($P = 0·99$). In analysis of all data, hierarchical linear modelling suggested that both gas type and pressure had no significant effect on renal blood flow ($P = 0·09$ and $P = 0·18$ respectively).

Hepatic artery blood flow

Mean hepatic artery flow was 17·2(2·8) ml per min per 100 g hepatic tissue before the creation of pneumoperitoneum or a laparotomy wound. Insufflation with carbon dioxide or helium resulted in no significant change in blood flow within the hepatic artery despite a wide range of

Table 2 Hepatic artery blood flow measured in anaesthetized animals before and after laparoscopy or laparotomy

	Blood flow (ml per min per 100 g)		
Group	Before	After	*P**
12 mmHg helium	7·5(1·5)	12·8 (3·0)	0·122
12 mmHg carbon dioxide	22·1(8·9)	19·8(8·6)	0·443
8 mmHg carbon dioxide	22·2(6·2)	18·4(2·5)	0·448
4 mmHg carbon dioxide	14·5(2·6)	18·8(6·9)	0·701
Laparotomy	19·7(7·4)	13·1(4·4)	0·106

Values are mean(s.e.m.). **t* test

Table 3 Peritoneal blood flow measured in anaesthetized animals before and after laparoscopy or laparotomy

	Blood flow (ml per min per 100 g)		
Group	Before	After	*P**
12 mmHg helium	3·0(1·9)	3·0(0·4)	0·99
12 mmHg carbon dioxide	5·0(2·9)	8·2(2·9)	0·002
8 mmHg carbon dioxide	2·3(0·4)	4·8(0·3)	0·001
4 mmHg carbon dioxide	1·7(0·2)	4·1(0·5)	0·065
Laparotomy	2·1(0·3)	3·6(0·6)	0·027

Values are mean(s.e.m.). **t* test

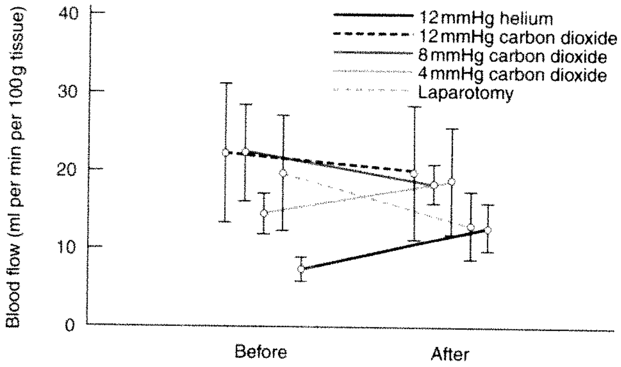

Fig. 2 Mean(s.e.m.) hepatic artery blood flow measured in anaesthetized animals before and after laparoscopy or laparotomy

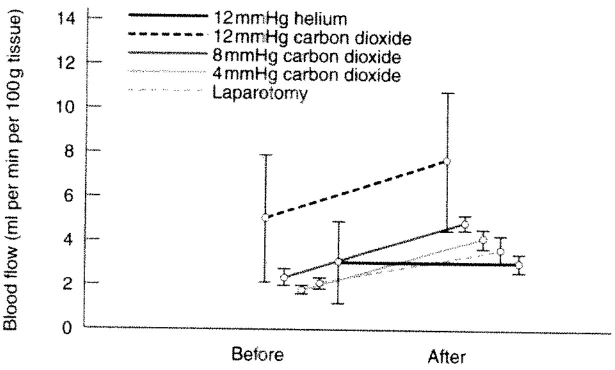

Fig. 3 Mean(s.e.m.) peritoneal blood flow measured in anaesthetized animals before and after laparoscopy or laparotomy

insufflation pressures (*P* = 0·54 for analysis of all insufflation pressures). Laparotomy produced no significant blood flow changes in the hepatic artery (*Table 2* and *Fig. 2*).

Peritoneal blood flow

Mean peritoneal blood flow was 3·7(0·5) ml per min per 100 g peritoneal tissue before the creation of pneumoperitoneum or a laparotomy wound. Blood flow to the peritoneum was significantly increased both by a laparotomy incision (*P* = 0·027) and by insufflation with carbon dioxide at 8 mmHg (*P* = 0·001) and 12 mmHg (*P* = 0·002) (*Table 3* and *Fig. 3*). However, the greatest peritoneal blood flows resulted from the insufflation of carbon dioxide at 12 mmHg. It is possible that the small sample size of group 4 (4 mmHg carbon dioxide) contributed to the non-significant difference observed with this insufflation pressure, as the rest of the data for carbon dioxide pneumoperitoneum clearly suggested a marked trend for increased peritoneal blood flow with carbon dioxide insufflation; alternatively a pressure of 4 mmHg was too low to induce significant alterations in peritoneal blood flow. Blood flow to the peritoneum was unchanged when helium was used as the insufflating gas at a pressure of 12 mmHg (*P* = 0·99).

Discussion

Laparoscopic surgery produces many benefits following colorectal resection, including less impaired respiratory function[11], reduced pain[12–16], a quicker recovery[16–19] and better cosmesis[20]. However, the learning curve is undoubtedly longer than that for other laparoscopic procedures such as laparoscopic cholecystectomy[2,21] and potentially the risk of wound (or port site) metastases is higher[22]. Most patients undergoing laparoscopic or laparoscopically assisted resection of a colorectal malignancy are therefore currently enrolled in trials or have laparoscopic procedures performed for palliation.

Current surgical literature suggests that the direct spread of viable tumour cells to the wound sites is an important mechanism in the aetiology of port-site metastases. However, it is not clear whether this is the result of laparoscopic surgery causing increased dissemination of viable cells[23–26] or the result of physiological changes induced by insufflation, which potentiate the development of such metastases.

Carbon dioxide is absorbed across the peritoneum[27], leading to a localized increase in its partial pressure. This may produce reflex vasodilatation and thereby increase blood flow to tumour cells at the port sites. This mechanism was proposed to occur in laparotomy wounds as far back as the nineteenth century[28].

Previous work has clearly demonstrated that insufflation pressures can have a profound effect on renal blood flow. London *et al.*[29] demonstrated that prolonged insufflation with carbon dioxide decreased renal blood flow by a maximum of 30 per cent and Shuto *et al.*[30] recorded a 74 per cent fall in renal blood flow in pigs subjected to a 20-mmHg insufflation, with similar results from both carbon dioxide and helium insufflation. However, it has also been shown that insufflation may have little effect on blood flow to intra-abdominal organs at insufflation pressures below 10 mmHg[31].

In the present study renal blood flow fell at all insufflation pressures with carbon dioxide (and following laparotomy), but this decrease was statistically significant only in the group undergoing insufflation at 8 mmHg. However, this may be related to the relatively shorter length of time under anaesthesia in this study than in previous work. It is also notable that, although readings were consistent between left and right kidneys, marked variation occurred between baseline renal blood flows; the variance was 4·9 per cent for all data, predominantly owing to interpig differences, and such differences with the small number of animals in each group may have masked some of the effect of insufflation. This potential source of error may have been reduced by measurement of cardiac output, permitting better standardization by allowing correlation with the proportion of cardiac output reaching the kidneys and peritoneum.

Liver blood flow has been shown to decrease with carbon dioxide and helium insufflation[30], more so with helium than carbon dioxide[27]. In the present model, insufflation gas pressures of 12 mmHg or less produced no change in hepatic artery blood flow. It is likely that the diminution in total hepatic blood flow is related to changes within the portal circulation; indeed, several reports have demonstrated that pneumoperitoneum reduces portal venous blood flow[32,33].

Marked changes occurred to peritoneal blood flow during carbon dioxide insufflation; an increase was demonstrated at all insufflation pressures, but peritoneal blood flow was unaffected by helium insufflation at 12 mmHg. It is therefore likely that the observed increases in peritoneal blood flow occurred as a result of local metabolic effects of carbon dioxide gas. This may partly explain the observed decrease in port-site recurrence rates in animal models when helium insufflation is compared with carbon dioxide insufflation[34,35].

If current clinical studies confirm the safety of helium insufflation, it is suggested that this gas should be considered for insufflation during laparoscopic resection of malignancies.

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Strategy for the surgical management of patients with idiopathic megarectum and megacolon (*Br J Surg* 2001; 88: 1392–6)

Sir

There are several points that warrant discussion. First, variable criteria were used to make the diagnosis of mega-bowel and although the definition is not standardized[1,2] all patients within a surgical outcomes study should satisfy the inclusion criteria.

Second, the choice of surgical procedure is guided by proximal colonic function, as assessed by perfused-tube manometry. The authors suggest that this has been validated in patients with post-childbirth constipation, although one of the references relates to constipated children[3]. The use of thin-bore catheters in a large cavity viscus will underestimate and misinterpret phasic activity[4]. Management based on such results may be misguided. More appropriate methods include the intraluminal barostat[5] or colonic scintigraphy[6].

Finally, we were surprised that vertical reduction rectoplasty[7] was not mentioned as an additional option. Although this procedure is not fully evaluated, early results have been promising with improvements in clinical and physiological function[7]. As the authors suggest, the results of restorative proctocolectomy, panproctocolectomy and proctectomy for this indication are mixed and, as in our study, limited to few patients. Proctectomy is a more radical pelvic procedure associated with significant morbidity and mortality, including disturbance of sexual and bladder function[8]. It would be of interest to know, therefore, whether the authors came across these complications in any of their patients.

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Suspected acute appendicitis: trends in management over 30 years (*Br J Surg* 2001; 88: 1570–7)

Letter 1

Sir

Localized pain and tenderness in the right suprapubic region is common in parous women with a history of prior difficult intrapartum episodes. Premature or prolonged maternal voluntary efforts are associated with avulsion of the origin of the levator ani from the arcus tendineus levator ani over obturator internus[1]. Subsequent scarring or reinnervation may give rise to the symptom of pain in the right lower quadrant and the physical sign of localized tenderness in the right suprapubic region. Bilateral injury may occur though unilateral right-sided injury is more frequent. The injury may be a consequence of the mechanics of vaginal delivery and the sigmoid colon may afford relative protection to the left side of the pelvis. Some peritoneal neovascularization may be noted on the right side of the pelvis at laparoscopy though this is non-specific and ill-defined. It seems unlikely that localized pain and tenderness would provide the sole basis for a decision to perform an appendicectomy but a careful history of prior intrapartum episodes may avoid imprecision in unusual presentations. These limited, gynaecological remarks are offered to complement Professor Jones' policy of 'active observation' as the avulsion injury is clearly non-progressive, and may be the source of much 'non-specific' abdominal pain in parous women.

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1 Quinn MJ, Slack MC. Anatomy of Denonvilliers' fascia and pelvic nerves, impotence, and implications for the colorectal surgeon. *Br J Surg* 2001; **88:** 888 (Letter).

Letter 2

Sir

Professor Jones has provided an excellent distillation of publications that explore the perennial problem of discriminating appendicitis from acute non-specific abdominal pain. His review deals with the respective values of computed tomography, ultrasonography, laparoscopy and active observation. May I recommend the abdominal wall tenderness test[1]. In doubtful cases a positive test has allowed most of my patients to be safely discharged without recourse to further investigation.

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1 Thomson H, Francis DM. Abdominal-wall tenderness: a useful sign in the acute abdomen. *Lancet* 1977; **2:** 1053–4.

An unexpected liver secondary (*Br J Surg* 2001; 88: 627–8)

Sir

It is cumbersome to perform intraoperative ultrasonography through a midline incision. Since intraoperative ultrasonography visualizes and characterizes lesions[1], we use the laparoscopic probe during open procedures and pathological confirmation is unnecessary. Moreover, in our series we have treated two patients with peritoneal seeding after liver biopsy, rendering them without options for curative treatment[2,3]. If a synchronous lesion is found, it is essential to exclude distant metastases before embarking on unnecessary procedures. Although liver surgery is associated with a low morbidity and mortality rate when compared with colorectal surgery we feel that the statement 'it is unlikely that opening the bowel increases the likelihood of infectious complications' cannot be backed up by the literature.

If a lesion is found in the liver, complete staging is mandatory. New therapeutic modalities such as radiofrequency ablation in combination with resection may provide a patient with the best chances for survival. Computed tomography and magnetic resonance imaging with positron emission tomography improves patient selection for liver resection[4]. The discovery of metastasis at the time of anterior resection is no reason to stop the procedure, unless there are massive liver metastases. Resection of a rectal carcinoma is still the best palliation.

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A normal appendix found during diagnostic laparoscopy should not be removed (*Br J Surg* 2001; 88: 251–4)

Sir

In this paper 14 per cent of patients presented during follow-up with abdominal pain and the assessing surgeon had to rely on hospital notes or the patient's memory to determine whether or not the appendix had been removed previously.

Since 1998 we have adopted a selective approach (normal appearing appendix left *in situ* at laparoscopy) but we were concerned about the reliability of patient recollection. We interviewed 176 patients who had undergone laparoscopy for abdominal pain during a 3-year period by telephone[1]. In response to the question 'Was your appendix removed at operation?', 93 per cent of those who answered 'yes' were correct but 61 per cent of those who answered 'no' were incorrect (their appendix had been removed). Those in whom no abnormality was found were significantly more likely to be confused or incorrect (61 per cent) than those in whom appendicitis was diagnosed (17 per cent).

We now believe that the removal of a normal appendix in a patient already exposed to most of the morbidity associated with appendicectomy (general anaesthesia, port placement, laparoscopy and visceral manipulation) adds little to the overall morbidity. The case for overturning over a century of tradition is far from proven and we propose that, once the patient is explored, the appendix should still be removed even if it appears normal at laparoscopy.

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1 Murphy SM, Colgan G, O'Neill S, Tanner WA, Keane FBV. Previous laparoscopy in abdominal pain – confusion or clarification. *Ir J Med Sci* 2001; **170:** 28 (Abstract).

Authors' reply

Sir

Murphy *et al.* emphasize the importance of paying considerable attention to informing the patient on the operative procedure after laparoscopic appendicectomy. We stress that in case of further abdominal complaints the patient must inform the attending physician that the appendix has been removed.

Removal of a normal appendix does not end the misconception by many patients that the appendix is still in place. The added risk of a 'prophylactic' appendicectomy may be low but not negligible, as described in an as yet unpublished study concerning the implications of removing a normal appendix (C. L. Bijnen, W. T. van den Broek, A. B. Bijnen, P. De Ruiter, D. J. Gouma, unpublished data).

Appendicectomy during diagnostic laparoscopy for abdominal pain should be considered an appendicectomy *en passant* and therefore, in our opinion, *passé*.

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'Fast track' postoperative management protocol for patients with high co-morbidity undergoing complex abdominal and pelvic colorectal surgery (*Br J Surg* 2001; 88: 1533–8)

Sir

We agree that shortening the length of hospital stay for patients undergoing colorectal surgery is worthwhile but were not satisfied that the authors' conclusions were relevant to the majority of colorectal practice. They proved neither the safety nor the worth of the multimodal care pathway used in this study. The patient population was unusually young (median age 44·5 years). Most colorectal surgery is performed on elderly patients for whom domestic circumstances delay discharge; our last 195 patients who underwent colorectal resection had a mean age of 67·9 years. We are not told the average length of stay in the hospital for such a young group before the trial. These patients may have gone home at 4–5 days anyway. Did the four readmissions and 12 early complications represent safety? The study required a traditionally managed control group for comparison. We were confused by the use of eight patients labelled as 'poorly compliant with the protocol' as a separate group for comparison with those who did comply, when the definition of the study group included those who stayed in hospital longer than deemed necessary by the team. This group stayed longer, by definition. Unless this subgroup could be identified before the protocol begins, the subdivision is irrelevant.

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Authors' reply

Sir

Our study addressed the application of 'fast track' pathways for reoperative abdominal and pelvic surgery, not only standard colon resection. The results mirrored those seen with less complex surgery in other studies of 'fast track' pathways performed in patients of approximately 65 years of age[1–3], suggesting that these results should be applicable to the practice of Rix and Jourdan. The patient age reflects the practice of this single-surgeon study, and the results for the last 2526 patients by the surgeon, and 11 241 patients in the department over the last decade are given in *Table 3*. The readmissions and complications described in the paper are low when compared with similar studies[1–3]. We have also recently completed a randomized controlled trial of this pathway and the results will be forthcoming shortly.

The overall results were expressed by intention-to-treat. 'Poorly compliant' patients were prospectively identified to explain a subgroup of patients that have a slower recovery, as in the Methods section. Further ongoing work, including the randomized trial, is designed to predict such subgroups preoperatively. We would contend that the objective support for this pathway is that this complex patient cohort had a 4·3-day postoperative stay.

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1 Kehlet H, Mogensen T. Hospital stay of 2 days after open sigmoidectomy with a multimodal rehabilitation programme. *Br J Surg* 1999; **86**: 227–30.
2 Di Fronzo LA, Cymerman J, O'Connell TX. Factors affecting early postoperative feeding following elective open colon resection. *Arch Surg* 1999; **134**: 941–5; discussion 945–6.
3 Bradshaw BG, Liu SS, Thirlby RC. Standardized perioperative care protocols and reduced length of stay after colon surgery. *J Am Coll Surg* 1998; **186**: 501–6.

Prevention of the neoplastic progression of Barrett's oesophagus by endoscopic argon beam plasma ablation (*Br J Surg* 2001; 88: 1357–62)

Sir

It is important to study innovative techniques that may prevent the malignant progression of Barrett's oesophagus. The authors claim that argon beam plasma coagulation can be used for the 'prevention of the neoplastic progression' of Barrett's oesophagus. Doubts regarding this claim remain in their discussion where they 'presume' a 'likely reduction' in the malignant progression. The claim of proof of therapy by 'lack of any malignancy' in patients with high-grade dysplasia is misleading. Their expectation to find five or six patients with malignant progression during the follow-up of 173·5 patient years is a significant overestimate as the group with highest risk of progression (i.e. high-grade dysplasia) had the shortest follow-up of only 20·8 months. Indeed, the treatment would have to be considered to be ineffective since at least 30 per cent had persistent intestinal metaplasia after treatment. Should there be concern about adding the insult of thermal injury to mucosa which has already undergone damage from acid and bile reflux? Complicated Barrett's oesophagus with ulceration or stricture formation may be at higher risk of malignant transformation[1], and intuitively this group might be expected to behave similarly. The prevention of adenocarcinoma of the oesophagus is of great importance but we must be cautious about adopting a therapy with a mortality rate of 3·6 per cent, especially in patients with uncomplicated Barrett's oesophagus. We would caution the authors' enthusiasm for this technique which they demonstrate is neither safe nor effective.

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1 Pera M, Trastek VF, Carpenter HA, Allen MS, Deschamps C, Pairolero PC. Barrett's esophagus with high-grade dysplasia: an indication for esophagectomy? *Ann Thorac Surg* 1992; **54:** 199–204.

Author's reply

Sir

We do not claim that argon beam plasma coagulation has been proven to prevent neoplastic progression in Barrett's oesophagus. The conclusion of our paper is that a randomized clinical trial is warranted. From the calculation of the total number of patients and those with high-grade dysplasia it is appropriate to say that five or six patients would have been expected to have malignant progression. Therefore, the lack of malignancy does give an indication that argon beam therapy might prove useful. In the 55 patients with a further 6-month follow-up, there is still no malignancy (total 320 patient years). Immanuel *et al.* have also quoted our mortality rate inaccurately as being 3·6 per cent. One death occurred in 55 patients. The patient had endoscopic ablative therapy for high-grade dysplasia because he was unfit for resection. The patient was keen for open surgery to remove the oesophagus once perforation occurred, but he died following thoracotomy. The technique of argon beam plasma coagulation is safe, with no perforations in the last 300 applications in our clinical service. A randomized clinical trial comparing argon beam therapy with observational therapy is justified to see if progression to malignancy in Barrett's oesophagus can be prevented.

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Extent of ductal carcinoma *in situ* within and surrounding invasive primary breast carcinoma (*Br J Surg* 2001; 88: 1324–9)

Sir

This work introduces the concept of closer examination of the normal tissue surrounding the tumour. We know that there is a 20–60 per cent chance of residual cancer in patients undergoing wide local excision[1,2] and this depends on the margin status. In how many patients, therefore, were the margins close or involved, and how far from the margin were the *in situ* cells?

There are several other significant questions: (1) Was this a prospective study or a retrospective examination of excised specimens? (2) What was the association between the extensive *in situ* component and ductal carcinoma *in situ* in the surrounding normal tissue? (3) Did the authors perform a multivariate analysis and find ductal carcinoma *in situ* in the normal tissue to be as, or more predictive of an adverse outcome compared to other known prognostic indicators?

Larger studies[3] have failed to show an increase in the incidence of metastasis and a reduced overall survival in patients undergoing wide local excision as compared to mastectomy.

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1 Wazer DE, Schmidt-Ullrich RK, Schmid CH, Ruthazer R, Kramer B, Safaii H *et al.* The value of breast lumpectomy margin assessment as a predictor of residual tumor burden. *Int J Radiat Oncol Biol Phys* 1997; **38**: 291–9.
2 Beck NE, Bradburn MJ, Vincenti AC, Rainsbury RM. Detection of residual disease following breast-conserving surgery. *Br J Surg* 1998; **85**: 1273–6.
3 van Dongen JA, Voogd AC, Fentiman IS, Legrand C, Sylvester RJ, Tong D *et al.* Long-term results of a randomized trial comparing breast-conserving therapy with mastectomy. European Organization for Research and Treatment of Cancer 10801 trial. *J Natl Cancer Inst* 2000; **92**: 1143–50.

Authors' reply

Sir

This was a retrospective study, principally directed at the clinical significance of the extent of ductal carcinoma *in situ* surrounding an invasive carcinoma and was not a study examining risk factors for local recurrence following conservation treatment of breast carcinoma. Margin status is a key risk factor for recurrence following breast conservation, but there is good evidence that routine pathological examination of margins and measurement of distance to margins is relatively inaccurate[1]. The extent of ductal carcinoma *in situ* in the surrounding breast tissues can increase the risk of margin involvement. In many specialist centres high-resolution magnification mammography is used to identify the presence of surrounding ductal carcinoma *in situ* and, if extensive, patients are often advised against breast conservation.

The aim of our study was to try to improve the understanding of the clinical relevance of the extent of ductal carcinoma *in situ* around and within invasive carcinomas with the long-term goal of developing effective strategies for routine classification. There are currently no clear guidelines, or indeed an international consensus, on the assessment of the extent of ductal carcinoma *in situ* in this situation.

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1 Silverstein MJ. Predicting local recurrences in patients with ductal carcinoma *in situ*. In: Silverstein MJ, ed. *Ductal Carcinoma In Situ of the Breast*. Philadelphia, Pennsylvania: Williams & Wilkins, 1997.

Randomized clinical trial comparing suture and mesh repair of umbilical hernia in adults (*Br J Surg* 2001; 88: 1321–3)

Letter 1

Sir

I would be interested in knowing the specific recurrence rates for those hernias over 3 cm in diameter repaired with mesh *versus* suture. Hesselink *et al.*[1], in a large retrospective review, found only a defect size of over 4 cm in diameter as a significant risk factor for recurrence. It has been my policy to repair all abdominal wall defects over 4 cm in diameter, or that have recurred, with a laparoscopically placed intra-abdominal mesh. The recurrence rates with this approach are low[2]. The findings of Arroyo *et al.* add to the growing body of evidence supporting the routine use of prosthetic mesh in all but the most trivial abdominal wall defects.

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1 Hesselink VJ, Luijendijk RW, de Wilt JH, Heide R, Jeekel J. An evaluation of risk factors in incisional hernia recurrence. *Surg Gynecol Obstet* 1993; **176**: 228–34.
2 Heniford BT, Park A, Ramshaw BJ, Voeller G. Laparoscopic ventral and incisional hernia repair in 407 patients. *J Am Coll Surg* 2000; **190**: 645–50.

Letter 2

Sir

Arroyo *et al.* report suture repair recurrence rates of 10–30 per cent from the literature and 11 per cent for primary repairs. Of 393 primary umbilical hernias repaired by sutures alone we found a recurrence rate of 4 per cent[1]. The major concern with mesh repair is the risk of infection. Although the risk may be low, it seems inappropriate to recommend the use of mesh. Clearly trials of sufficient power are required to answer the questions of recurrence rate and infection.

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1 Bowley D, Kingsnorth A. Umbilical hernia, Mayo or mesh? *Hernia* 2000; **4:** 195–6.

Authors' reply

Sir

Despite the paradoxical results, the size of the hernia defect did not affect outcome. The differences in results seemed to be due to the technique (mesh *versus* suture repair) rather than hernia size. For defects greater than 3 cm in diameter the recurrence rate was 3 per cent (one of 32) in the mesh group compared to 13 per cent (four of 30) in the suture group. For smaller defects the recurrence rate was zero after mesh repair *versus* 10 per cent (seven of 70) in the suture group. These results were not reported separately in the paper, though we mentioned there was no effect of hernia size. This finding might fit with the philosophy of the tension-free repair, in which the hernia size may not play a decisive role in recurrence rates.

According to the literature, the risk of infection after mesh umbilical hernia repair may be as low as 1 per cent. We found wound infection in 2 per cent of patients in the study; none required removal of the mesh. Some 3 per cent of wounds in the suture group became infected but there was no statistical difference between the groups. A single dose of amoxycillin–clavulanic acid was administered preoperatively, although some authors have questioned the use of prophylactic antibiotics. We consider the 4 per cent recurrence rate supports the use of herniorrhaphy in repairing umbilical defects, but the results are not strong enough to reject the use of mesh on the grounds of infection risk.

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Prospective randomized double-blind placebo-controlled trial of glyceryl trinitrate in endoscopic retrograde cholangiopancreatography-induced pancreatitis (*Br J Surg* 2001; 88: 1178–82)

Letter 1

Sir

We have carried out a similar double-blind controlled trial[1] that resulted in a reduction both in the incidence of post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis and in delayed enzymatic increase at 24 h, but not at 2 h. We expect that the final and complete report will be published shortly. Sudhindran *et al.* did not find a difference in the extent of hyperamylasaemia at 6 h, but no information is provided about the level at 24 h. They administered 2 mg sublingual long-acting glyceryl trinitrate (GTN) to their patients which, according to the authors, has a sustained systemic action for 6 h. We used a 15-mg GTN patch, applied 30–40 min before the procedure. It has its peak action at 60–120 min and lasts for 24 h[2]. On the other hand, we based the diagnosis of pancreatitis on a four-fold enzymatic increase and a history of abdominal pain lasting more than 24 h. We selected this pharmacological method on the hypothesis that the post-ERCP pancreatitis is related to spasm and oedema of the sphincter of Oddi, more than to hyperpressure secondary to cannulation or to direct trauma. A significant reduction in post-ERCP pancreatitis and enzymatic increase at 24 h but not at 2 h seems to confirm the need for sustained pharmacological action.

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1 Moretó M, Zaballa M, Ramírez K, Urcelay R, Merino O, Casado I *et al.* Transdermal glyceryl trinitrate in the prevention of post-ERCP pancreatitis. *Gastrointest Endosc* 2000; **51**: AB67.
2 Micromedex Healthcare Series, Volume 109, Edition 9. Greenwood Village, Colorado: Micromedex, 2001.

Letter 2

Sir

It is known that the incidence of post-endoscopic retrograde cholangiopancreatography (ERCP)-induced pancreatitis is dependent on factors such as volume of the contrast used, technical difficulty of cannulation, young patient age and number of pancreatic contrast injections[1]. Further multiple cannulations and prolonged manipulation are also proven risk factors[2]. We were surprised that these issues seem to have been overlooked in the design of this study.

Did the authors note any technical difficulty in cannulation (although cannulation rates are the same in both groups) in the study group? If so, what criteria were use to grade the technical ease of cannulation of the papilla?

One of the most powerful risk factors for the development of pancreatitis is suspected dysfunction of the sphincter of Oddi[1,3]. Could sphincter dysfunction have contributed to the development of post-ERCP pancreatitis?

Finally, it would be interesting to know the rate of inadvertent cannulation of the pancreatic duct.

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1 Freeman ML, Nelson DB, Sherman S, Haber GB, Herman ME, Dorsher PJ *et al.* Complications of endoscopic biliary sphincterotomy. *N Engl J Med* 1996, **335**: 909–18.

2 Taransky P, Cunningham J, Cotton P, Hoffman B, Palesch Y, Freeman J *et al.* Pancreatic sphincter hypertension increases the risk of post-ERCP pancreatitis. *Endoscopy* 1997; **29**: 252–7.

3 Budzynska A, Marek T, Nowak A, Kaczor R, Nowakowska-Dulawa E. A prospective randomised placebo controlled trial of prednisone and allopurinol in the prevention of ERCP-induced pancreatitis. *Endoscopy* 2001; **33**: 766–72

Author's reply

Sir

We were unaware of the trial conducted by Moretó *et al.* using 15-mg glyceryl trinitrate (GTN) patches that have a much longer duration of action than the sublingual GTN used in our study. However, even with our dosage of GTN, more than 50 per cent of the treatment group experienced headaches and suffered with hypotension. It would be interesting to know the side-effect profile and tolerance of long-acting GTN. In our study there was no significant difference in the amylase levels at 12 or 24 h between the two groups.

We agree with Harinath and O'Riordan that there are a large number of procedure- and patient-related factors that may contribute to post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis. The study that they quote is a multicentre observational study rather than a prospective randomized double-blind study[1]. One of the common indications for ERCP in that study was sphincter of Oddi dysfunction. This is a controversial syndrome of unknown cause which is suspected, particularly in North America, as a cause of recurrent abdominal pain after cholecystectomy, chiefly in women. No patient in our study underwent ERCP for suspected sphincter of Oddi dysfunction. The other risk factors mentioned such as ease of cannulation, prolonged manipulation and multiple cannulations, are very subjective and difficult to quantify accurately. These are, however, closely related to the endoscopic technique, which is linked to the skill and training of the endoscopist[1]. In our study, a single experienced endoscopist performed all the procedures. This fact, together with proper randomization, should have almost completely eliminated any such imbalance between the two groups. The rate of inadvertent pancreatic duct cannulation was similar in both groups as well (7 per cent in the GTN group and 8 per cent in the placebo group).

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1 Freeman ML, Nelson DB, Sherman S, Haber GB, Herman ME, Dorsher PJ *et al* Complications of endoscopic biliary sphincterotomy. *N Engl J Med* 1996; **335**: 909–18.

Bladder Cancer: Current Diagnosis and Treatment

M. J. Droller (ed.)

230 × 155 mm. Pp. 454. Not illustrated. 2001. Totowa, New Jersey: Humana Press. US$135·00.

This book presents a detailed summary of all aspects of bladder cancer and comprises contributions from 44 (principally American) experts in this field. The text is divided into 17 fully referenced, sequentially arranged chapters, covering issues from demographic and epidemiological aspects of the disease right through to treatment options for metastases. Ample coverage is given to the often confusing biological and molecular aspects of the development and progression of bladder cancer, providing an excellent link between basic sciences and clinical practice. Extensive discussion is given to the diagnosis of bladder cancer, with a useful guide to the pitfalls in staging that is invaluable for those learning the nuances of managing this disease in the clinical setting. Two very elegant chapters by Mark Soloway and Michael O'Donnell summarize the extensive literature on intravesical therapy. Both Donald Skinner and Arnulf Stenzl contribute to the surgical approaches to invasive disease. Overall, this text provides a precise synopsis of all contemporary issues in the management of bladder cancer. It would be useful as a reference to a busy urologist in practice and provides a well summarized, manageable text for those trainees sitting the Fellowship of the Royal College of Surgeons (Urology) examination.

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Mastery of Surgery. 4th Ed.

R. J. Baker and J. E. Fischer (eds)

285 × 215 mm. Pp. 2448. Illustrated. 2001. Philadelphia, Pennsylvania: Lippincott Williams and Wilkins. US$359·00.

This large tome, in two volumes, provides a very comprehensive account of anything that could possibly be described as 'general surgery'. Written by a large number of acknowledged experts, it is at its best when describing surgical techniques, although there is also much on perioperative care and patient selection. If you want to find out how a particular well known expert approaches an operation, this book is an ideal starting point. The descriptions of operative procedures are detailed and clear, and there is good use of line drawings. It is actually refreshing to see such clear black and white illustrations. Opinions are clearly expressed throughout and there are also extensive editorial comments. Paradoxically, this is simultaneously the major strength of the book and its major weakness. Many opinions are backed up by good argument, good reviews and appropriate evidence, but there are still examples of the repetition of evidence-free mantras. Some sections, such as that on peptic ulcer disease, have a rather old fashioned air, but overall the book is up to date. There is nothing else quite like it on the market.

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Essential General Surgical Operations

R. M. Kirk and M. Winslet

275 × 219 mm. Pp. 273. Illustrated. 2001. London: Churchill Livingstone. £31·95.

This book is adapted from the larger parent text *General Surgical Operations* and aims to focus the basic surgical trainee on those operations that are important for the membership of the Royal College of Surgeons examination. Although the reader is referred in the preface to other works for perioperative management, the text comprehensively deals with the entire perioperative process, from preparation of the patient to management of important post-operative problems. A strong point is the inclusion of gems of practical wisdom that are rarely mentioned in books of this kind, information that may become apparent in day-to-day clinical practice but which may be thought of as self-evident by trainers.

Each procedure is usefully divided into sections and well illustrated with line drawings. Chapters on issues related to surgery, such as endoscopy and anaesthesia, are included. While all surgical specialties are entertained, the book deals only with those operations relevant to the basic surgical trainee. Unfortunately, there are frequent references to certain procedures or instruments by name but no elaboration, e.g. types of proctoscopes (Eisenhamer, Parks, Sims) with no diagram to distinguish them. Diagrams are also lacking in some areas and this would have reduced the need for text. A chapter on types of surgical instruments and sutures would have been helpful but the author does begin by stating that this is a 'what to' rather than a 'how to' book. This is a clear, concise and practical text, and the first chapter 'Choose well, cut well, get well' sets an appropriate theme. As a basic surgical trainee I found this a useful volume and I would happily recommend it to fellow trainees.

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Hamilton Bailey's Emergency Surgery. 13th Ed.

B. W. Ellis and S. Paterson-Brown (eds)
278 × 203 mm. Pp. 804 Illustrated 2000. London: Arnold. £95·00.

This is certainly not a textbook for the on-call registrar's pocket, but it is a well designed comprehensive guide to emergency surgery. In addition to the traditional approaches to most emergencies, it contains some bonuses and little gems. The chapter on urban violence is useful and the one on medicolegal aspects of emergency work is interesting in light of the current climate. I should have liked more chapters with suggested extended reading, but I was impressed to see modern technology catching up with the printed word with the inclusion of the occasional website address. The book is attractive in these days of increasing specialization; having an idea of how other specialties deal with emergencies in the face of multiply injured patients may help in improving one's prioritization in the emergency room. The breadth of information contained in the volume (including tropical emergencies) will give it worldwide appeal, but as travel increases more and more of these problems may be seen in your local casualty department. On the downside is the limited use of authoring, which is especially notable in the specialist sections. I think the biggest question is whether or not 60 pages of orthopaedic emergencies is a worthwhile inclusion, as there are specialist acute orthopaedic texts available that are considerably more comprehensive. Overall, this is a worthwhile addition to the library of any surgeon involved in emergency patient care; however, it is something to be read in the cold light of day and not in the emergency room.

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Operative Pediatric Urology. 2nd Ed.

J. D. Frank, J. P. Gearhart and H. M. Snyder III (eds)
280 × 220 mm Pp 292 Illustrated. 2001 Edinburgh Churchill Livingstone. £94·95.

The first edition of this beautifully illustrated textbook was published in 1990, and edited by David Frank and Herbert Johnston. The American spelling of the word paediatric is presumably partly due to the fact that two new American editors have taken the place of Mr Johnston. Most of the chapters have been rewritten, and several new authors have been included. Each chapter consists of a concise description of an operation, some of which would be performed only by highly trained paediatric urologists with subspecialty interests. The outstanding illustrations by Philip Wilson ensure that every step of the described operation is easily understood; they are better than photographs. Three chapters have excellent drawings by Gillian Oliver. The book is written by experts; each author is able to give not only a detailed description of technique, but an insight into the potential problems. The newly added chapters, for example those on gastric augmentation and continent urinary diversion, are excellent, and help to keep the reader up to date with some of the innovations in paediatric urological surgery. It was also pleasing to see that chapters such as the one on ureteric reimplantation, by (a now retired but ever respected) Joe Cohen, are still included. In the preface, the authors do not state precisely at whom this book is aimed, but anybody with an interest in paediatric urology will find this essential reading. Trainees preparing for the specialist examination in urology are especially advised to read it.

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Lung Volume Reduction Surgery

M. Argenziano and M. E. Ginsburg (eds)
250 × 190 mm Pp 273 2001 Totowa, New Jersey: Humana Press
US$145 00

In the last 6 years there has been an explosion of interest in the surgery of emphysema, and in the operation that has become known as lung volume reduction surgery (LVRS). This attractive and handy-sized book covers this subject comprehensively, with fully referenced contributions from all of the multidisciplinary team required for such major surgery to prove safe and effective in a severely compromised group of patients. The 16 chapters are grouped, somewhat artificially, into two sections. The first covers, in six chapters, the pathogenesis, pathophysiology, radiology and medical management of emphysema; there is an excellent contribution on pulmonary rehabilitation, now receiving renewed attention in the UK. The second part, in ten chapters, covers the surgical aspects and controversies surrounding open *versus* thoracoscopic approaches, the improvement after surgery and its durability, concurrent surgery for lung cancer and emphysema, and the interface of transplantation with LVRS. Individual members of any team involved in LVRS will enjoy reading this book, but will probably choose selected chapters related to their prime area of involvement. The publishers and authors are to be congratulated in 'seizing the moment' and producing a textbook that covers this evolving subject without being obsolete at publication.

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Medicolegal Reporting in Orthopaedic Trauma. 3rd Ed.

M. Foy and P. Fagg (eds)
250 × 190 mm. Pp. 541. 2001. London: Churchill Livingstone. £99·95.

This third edition has been refined in both appearance and content from the ring binder of a decade ago. Foy and Fagg still do a very good job, covering the whole musculoskeletal system and a bit more (the head and a couple of lawyer's chapters). There is no doubt that matters to do with the spine are greatly over-represented in the medicolegal world (compared with the limbs), but in Foy and Fagg the spine is distillated down to not much more than 50 pages. I certainly seem to go to court much more often than my appendicular skeletal colleagues, but perhaps this is because some orthopaedic surgeons still hold the pre-Galilean view that disc hernias are in some way traumatic while others hold the opinion that whiplash is a virtually fatal condition. Arms and legs are much more predictable in their response to trauma and so in the next edition (and there certainly will be one because this is an excellent book) I would like to see more about the spine. There is a lot of factual information available and the more that is presented, the less variable opinion will be. I thought to begin with that there was too much Foy and Fagg, in that mushrooming subspecialization makes it difficult to be a credible author about so many different anatomical parts, but what they do they do very well indeed. The first four chapters about the medicolegal process from both an orthopaedic and legal point of view is a particularly good start, and is a worthwhile read for those embarking on a medicolegal career. There then follow sections on upper and lower limb fractures and miscellaneous topics (such as repetitive strain injury, psychological effects of trauma, and radiology in medicolegal practice). The non-spinal text adds up to a huge 350 pages.

The comprehensive reference lists at the end of each chapter are extremely valuable. For important detail and to be a real 'expert' witness, one must go to the individual papers cited. For example, did you know that 40 per cent of patients in group 3 (the severe group) in Norris and Watt's often quoted paper on prognosis following whiplash injuries had actually broken their necks or had Klippel–Feil syndrome – jolly good reasons for associated neurological abnormalities and long-term problems. None the less, those in group 3 were off work only for an average of 10 weeks. Now we have an epidemic of cases and an even bigger epidemic of papers, most of them no longer even bothering to take into account the force vector from behind, and lumping together any old soft tissue neck strain as a 'whiplash-associated disorder'. Meanwhile, the condition might well be nomogenic, as evidenced by the incidence dropping by 60 per cent if one must tell the police about the accident and bear the first few dollars of expense oneself. It does not exist in the indigenous Singaporians, only in 'ex-pats'; nor does it exist in Eastern Europe. Anyway, back to Foy and Fagg: a unique collection of information and well worth the money at 18 pence per page.

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Scientific Surgery

The *BJS* is committed to the practice of surgery based on scientific evidence. Each month we will publish a list of randomized trials and meta-analyses collated from English-language publications. A collection of all these papers will be available in the Scientific Surgery archive which will be held on the *BJS* website together with links to free Medline sites where the full papers can be accessed (see below for details). If you have recently published a randomized trial that has not been featured in Scientific Surgery, the Editors would be pleased to receive a reprint and consider its inclusion.

Rice HE, Brown RL, Gollin G, Caty MG, Gilbert J, Skinner MA *et al.* Results of a pilot trial comparing prolonged intravenous antibiotics with sequential intravenous/oral antibiotics for children with perforated appendicitis. *Arch Surg* 2001, **136:** 1391–5.

The clinical success rates were similar for both treatment regimens, but only 26 children were included

Yilmaz S, Kirimlioglu V, Katz DA, Kayaalp C, Caglikulekci M, Ara C. Randomised clinical trial of two bypass operations for unresectable cancer of the pancreatic head. *Eur J Surg* 2001; **167:** 770–6.

In this trial including 44 procedures there was a trend towards better clinical results with isoperistaltic *versus* antiperistaltic gastroenterostomy.

Wong JC, Torella F, Haynes SL, Dalrymple K, Mortimer AJ, McCollum CN; The ATIS Investigators. Autologous *versus* allogeneic transfusion in aortic surgery: a multicentre randomized clinical trial. *Ann Surg* 2002; **235:** 145–51.

All clinical outcomes and complications were similar in this study which included 145 procedures. An autologous transfusion policy reduced the median volume of transfusion and the total number of patients who required blood transfusion from 56 to 43 per cent.

Neoptolemos JP, Dunn JA, Stocken DD, Almond J, Link K, Beger H *et al.* Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. *Lancet* 2001; **358:** 1576–85.

Some 541 patients were included in this multifactorial trial design, there was a survival advantage from adjuvant chemotherapy (median 19 7 *versus* 14·0 months, P = 0 0005), but not from additional radiotherapy.

King M-C, Wieand S, Hale K, Lee M, Walsh T, Owens K *et al.* Tamoxifen and breast cancer incidence among women with inherited mutations in *BRCA1* and *BRCA2*. National Surgical Adjuvant Breast and Bowel Project (NSABP-P1) Breast Cancer Prevention Trial. *JAMA* 2001; **286:** 2251–6.

Within a randomized trial of breast cancer prevention with tamoxifen, 19 women with *BRCA* genetic mutations who developed breast cancer were examined Tamoxifen reduced the rate of cancer by 62 per cent, similar to the rate for all women in the trial.

Bartelink H, Horiot J-C, Poortmans P, Struikmans H, Van den Bogaert W, Barillot I *et al.* Recurrence rates after treatment of breast cancer with standard radiotherapy with or without additional radiation. *N Engl J Med* 2001; **345:** 1378–87

In patients with early breast cancer who had breast-conserving surgery, additional radiotherapy to the tumour bed reduced the risk of local recurrence (hazard ratio 0 59, 95 per cent confidence interval 0·43 to 0 81), especially in patients under 50 years old

Flanigan RC, Salmon SE, Blumenstein BA, Bearman SI, Roy V, McGrath PC *et al.* Nephrectomy followed by interferon α-2b compared with interferon α-2b alone for metastatic renal-cell cancer. *N Engl J Med* 2001; **345:** 1655–9.

The addition of nephrectomy to interferon prolonged median survival from 8 1 to 11 1 months (P = 0 05) in this study of 120 patients

Urschel JD, Urschel DM, Miller JD, Bennett WF, Young JE. A meta-analysis of randomized controlled trials of route of reconstruction after esophagectomy for cancer. *Am J Surg* 2001, **182:** 470–5.

Six randomized trials were evaluated, there was no difference between anterior or posterior mediastinal routes, but the number of procedures was small

Nenci GG, Gresele P, Ferrari G, Santoro L, Gianese F; Mesoglycan Intermittent Claudication Group. Treatment of intermittent claudication with mesoglycan – a placebo-controlled, double-blind study *Thromb Haemost* 2001; **86:** 1181–7.

Some 237 patients were analysed, walking distance increased by over 50 per cent in 50 per cent of those treated and in 26 1 per cent of the control group (P < 0 001).

Ishida H, Yokoyama M, Nakada H, Inokuma S, Hashimoto D. Impact of oral antimicrobial prophylaxis on surgical site infection and methicillin-resistant *Staphylococcus aureus* infection after elective colorectal surgery. Results of a prospective randomized trial. *Surg Today* 2001; **31:** 979–83.

Additional preoperative antibiotics reduced the rate of surgical site infection from 23·9 to 11·1 per cent (P = 0 04) in this study of 143 procedures

The *BJS* website can be found at *www.bjs.co.uk*. In addition to details of current and previous articles featured in Scientific Surgery, the website contains details of the Editorial Team and Board, instructions to prospective authors, links to other sites (such as The Cochrane Library) and forthcoming surgical events. The Editors welcome comments and suggestions about the website, either in writing or through the website address.

Society of Academic and Research Surgery

The following 93 abstracts were presented to the inaugural meeting of the Society of Academic and Research Surgery in January 2002. The meeting was held in the Sir Ian Fleming building at Imperial College in South Kensington, London, and was hosted by Professor A. Darzi and his colleagues at St Mary's Hospital, London. The Patey prize was won by R. J. Davies *et al.* for a paper entitled 'Novel stool-based screening test for colorectal cancer using antibodies directed against DNA replication protein, minichromosome maintenance protein 2'.

Transdermal oestrogen therapy for advanced prostate cancer

J. L. Ockrim, E. N. Lalani, M. E. Laniado, S. S. Carter and P. D. Abel (introduced by Professor A. Darzi)
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Background: Conventional hormone deprivation for advanced prostate cancer is associated with significant morbidity. Evidence suggests oestrogen therapy is equivalent to orchidectomy and maximal androgen blockade, but oral oestrogens were abandoned because of cardiovascular toxicity. By contrast, recent data suggest that parenteral oestrogens reduce cardiovascular morbidity by more than 90 per cent. This is the first report of transdermal (parenteral) oestrogens in men with advanced prostate cancer.

Methods: Local ethics committees approved the study. Twenty patients with locally advanced ($n = 10$) or metastatic ($n = 10$) prostate cancer were enrolled. Six transdermal oestradiol (7·8 mg) patches were applied weekly for 8 weeks, then adjusted to maintain castrate levels. Hormones, prostate-specific antigen (PSA), thrombophilic assays and vascular flow (to monitor for thrombosis) and quality of life (QoL) questionnaires were assessed at regular intervals. Disease progression and osteoporosis were assessed 6 monthly using computed tomography, bone scan and bone densitometry.

Results: Median follow-up was 10 (range 6–14) months. All patients had castrate levels of testosterone (less than $3\ \text{nmol l}^{-1}$) within 2 weeks and evidence of disease regression (PSA reduction averaged 95·1 (range 84·2–99·8) per cent). One patient had biochemical relapse at 14 months. No significant cardiovascular or vascular flow complications were recorded. Early data suggest that bone density is maintained. Grade I or II gynaecomastia occurred in 70 per cent of the men, but no hot flushes. All other QoL domains improved. Patches were well tolerated.

Conclusion: Transdermal oestrogens produce castrate levels of testosterone and are acceptable to patients, with minimal morbidity (gynaecomastia). Transdermal oestrogens protect against osteoporosis and hot flushes, improve QoL and are one-tenth the cost of current therapies, potentially saving the NHS more than £10 million annually.

Gastrin protects against the growth inhibitory effects of cytotoxic agents

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Background: Gastrin circumvents apoptosis in malignant cells. The aim of this study was to determine whether inhibition of the gastrin gene increases sensitivity to a panel of cytotoxic agents in gastrointestinal tumour cell lines.

Methods: The human gastrointestinal tumour cell lines PAN1 and HCT116 were stably transfected with an antisense construct to the gastrin gene. PAN1 was also transfected with a gastrin sense construct. Progastrin secretion was assessed by means of radioimmunoassay. *In vitro* proliferation of cell clones was measured with a tetrazolium-based colorimetric assay (five replicates per condition). The cytotoxic agents used included cisplatin, camphothecin and gemcitabine at concentrations ranging from 0·01 to $1\ \mu\text{g ml}^{-1}$. Apoptosis was assessed by use of an *in situ* DNA fragmentation kit (Oncogene).

Results:

	IC_{50} ($\mu\text{g ml}^{-1}$)		
	Vector control	Gastrin antisense	Gastrin sense
PAN1			
Gemcitabine	0·9	<0·01*	7·5†
Cisplatin	2·5	5·0*	4·0
Camphothecin	2·5	0·25*	30·0*
HCT116			
Cisplatin	7·0	3·0*	—
Camphothecin	70·0	25·0*	—

IC_{50}, inhibitory concentration at which 50 per cent of cells die. $^*P < 0·001$, $^†P < 0·05$ *versus* vector control (one-way analysis of variance)

Cell lines transfected with gastrin antisense had increased sensitivity to the antiproliferative effects of the cytotoxic agents. PAN1 cells transfected with gastrin sense had increased resistance. The increased inhibitory effect of the cytotoxics in the gastrin antisense cell lines was reversed by the addition of excess gastrin and 10 per cent serum-containing medium. Increased apoptosis was shown in the cisplatin-treated gastrin antisense transfected cells by the

DNA fragmentation assay. Progastrin concentration in PAN1 cells was 58 pmol l^{-1} in the vector control, not detected in the gastrin antisense cell line and 1220 pmol l^{-1} in the gastrin sense cell line.

Conclusion: Gastrin neutralization may increase sensitivity to cytotoxic agents.

Novel role for toll-like receptor 2 in human neutrophil-mediated bacterial phagocytosis

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Background: Invading bacteria are phagocytosed and killed by polymorphonuclear neutrophils (PMNs) through CR3 and Fcγ receptor II/III. Toll-like receptor (TLR) 2 has been recognized as a primary receptor for mediating intracellular signalling of bacterial lipoprotein and other bacterial cell wall components. Little is known regarding whether TLR-2 is involved in PMN-mediated bacterial phagocytosis and intracellular killing.

Methods: Human PMNs were obtained from healthy adult volunteers using dextran/Ficoll density sedimentation. Heat-killed *Staphylococcus aureus* and *Escherichia coli* were labelled with fluorescein isothiocyanate (FITC) and opsonized with pooled human sera. Bacterial binding to PMNs was assessed by flow cytometry. Bacterial ingestion by PMNs was analysed after the fluorescence of the bound but uningested bacteria had been quenched. Bacterial intracellular killing by PMNs was examined using live *S. aureus* and *E. coli*, and corrected with cytochalasin B treatment. PMN TLR-2 was totally blocked with a specific human TLR-2-blocking monoclonal antibody (Mab) 2392 (25 μg ml^{-1}).

Results: Blocking PMN TLR-2 did not affect bacterial binding to PMNs. PMN-mediated bacterial ingestion and intracellular killing of *S. aureus* and *E. coli* were both significantly inhibited when TLR-2 was blocked.

	Binding (MCF)	Ingestion (MCF)	Killing (%)
S. aureus			
PMNs	283(47)	106(15)	100(11)
PMNs + Mab	277(59)	35(10)*	36(8)*
E. coli			
PMNs	225(33)	72(12)	100(9)
PMNs + Mab	210(23)	41(9)*	67(6)*

Values are mean(s.d.). MCF, mean cell fluorescence. *$P < 0.05$ *versus* PMNs without Mab 2392 (analysis of variance)

Conclusion: These results indicate a crucial role for TLR-2 in initiating PMN-mediated phagocytosis and intracellular killing of invading micro-organisms during bacterial infection.

Potential of dietary supplementation in patients with carotid artery disease

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Background: Rupture of an atherosclerotic plaque is common and often leads to major clinical events such as stroke. Some polyunsaturated fatty acids (PUFAs), such as those in fish oil (n-3 PUFAs) and sunflower oil (n-6 PUFAs), have been suggested to improve plaque stability. The rapidity and extent to which dietary supplementation with these PUFAs altered plasma and atherosclerotic plaque lipid profiles were investigated.

Methods: Some 168 patients awaiting carotid endarterectomy were recruited to the study, which was approved by the ethics committee. Patients were randomized to take capsules containing n-3 PUFAs (group 1), n-6 PUFAs (group 2) or placebo (group 3). Plasma lipid profiles were determined before and after supplementation. Fatty acid composition and the concentration of KP-1, a marker of inflammation, in the plaque were determined after operation.

Results: Median duration of supplementation was 50 (range 7–94) days. Group 1 showed a significant change in lipid profiles: the plasma level of low density lipoprotein n-3 PUFAs increased from 1·2 to 4·2 g per 100 g total fatty acids. This was reflected by significantly increased n-3 PUFA plaque content in group 1 (1·45 *versus* 1·13 and 1·08 g per 100 g total fatty acid in groups 2 and 3 respectively). This was matched by a significant reduction in KP-1 concentration in group 1 compared with that in groups 2 and 3.

Conclusion: Short-term supplementation of fish oil (n-3 PUFAs) resulted in a rapid change in plasma and plaque lipid profiles. This suggests that it may be possible to affect atherosclerotic disease progress by dietary supplementation.

Inhibition of tumour xenograft growth by targeting hypoxic gene expression

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Background: A determinant of conventional cancer treatment failure and malignant progression is hypoxia within the tumour microenvironment. Hypoxia inducible factor (HIF) 1α, a transcription factor that mediates changes in gene expression, is a key element in the hypoxic cellular response. Two alternative cloning strategies were designed to suppress its activity.

Methods: Four cell groups were established for the colorectal (HT-29) cancer cell line: (1) wild type (unaltered cancer cell); (2) pcDNA3 transfected (an empty control vector); (3) HIF-1α dominant negative transfected; and (4) HIF-1α antisense transfected. HIF-1α dominant negative or antisense were the alternative strategies used to suppress HIF-1α activity. Forty-four nude mice were divided into four groups ($n = 11$) to correspond with the cell groups. Each animal received a subcutaneous injection of 1×10^6 cells into each flank. Animals were monitored for tumour development. Tumours were measured twice a week until death (day 24 after inoculation). Tumour growth was assessed by calculating the tumour volume using the standard equation $V = 0{\cdot}5\ (L \times W^2)$, where V is tumour volume, L is tumour length and W is tumour width.

Results: Transfection with HIF-1α antisense inhibited tumour growth. This effect was found to be significant at all time points, compared with wild-type and pcDNA3-transfected controls ($P < 0{\cdot}001$). Transfection with dominant negative HIF-1α produced a similar response but the effect was significant only on days 20 and 24 ($P = 0{\cdot}041$ and $P = 0{\cdot}005$ respectively). At death, tumours transfected with HIF-1α antisense or dominant negative were 43 per cent (mean(s.e.m.) 0·09(0·02) cm^3) and 24 per cent (0·12(0·026) cm^3) smaller in volume, respectively, than tumours transfected with control plasmid (0·16(0·02) cm^3).

Conclusion: These results demonstrate that targeting HIF-1α by either antisense or novel dominant negative protein strategy significantly inhibited tumour xenograft growth in nude mice and may have a bearing on cancer therapy development in the future.

Leucocyte–endothelium interaction in the symptomatic carotid plaque

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Background: Differences in the natural history of symptomatic and asymptomatic carotid stenosis may be reflected in changes in plaque biology.

Methods: Following ethics committee approval, carotid artery plaques were obtained from symptomatic ($n = 34$) or asymptomatic ($n = 10$) patients with internal carotid artery stenosis greater than 70 per cent. These were stained for the adhesion molecules intercellular adhesion molecule (ICAM) 1, P-selectin, E-selectin, platelet endothelial cell adhesion molecule (PECAM) and the gelatinase matrix metalloproteinase (MMP-9). Areas of staining for the whole plaque, cap, core and shoulder were analysed with the analySIS computer programme.

Results: The study comprised 44 patients of whom 32 were men (mean age 70·1 (range 50–83) years).

	Area of staining (μm^2)	
Total plaque	Symptomatic ($n = 34$)	Asymptomatic ($n = 10$)
PECAM	22·77 (18·66–38·72)	16·15 (8·83–27·37)
MMP-9	19·33 (12·62–24·78)	8·13 (5·18–15·3)†
ICAM-1	17·55 (0–22·48)	5·70 (0–13·56)*
P-selectin	6·60 (0–18·93)	0 (0–5·66)*
E-selectin	6·01 (0–18·44)	0 (0–7·82)

Values are median (interquartile range). $^*P = 0{\cdot}05$, $^\dagger P = 0{\cdot}006$ *versus* symptomatic (Mann–Whitney U test)

Conclusion: The levels of factors promoting leucocyte adhesion and migration are greatest in symptomatic carotid artery plaques and lowest in plaques from asymptomatic patients.

Detection of telomerase activity in circulating colorectal tumour cells

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Background: Despite apparently curative resection, up to 10 per cent of patients with Dukes' A colorectal cancer and 25 per cent of those with Dukes' B tumours relapse and die from metastatic disease within 5 years. This indicates that the metastatic process may already have been established before or at the time of resection, and that the detection of tumour cells in the circulation at this time may have important prognostic or diagnostic implications.

Methods: Thirty-six sequentially presenting patients undergoing surgery for colorectal cancer and ten healthy controls were included in the study. Patient blood samples were taken before operation, and at 1 and 7 days after surgery. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by Ficoll density gradient centrifugation. Circulating epithelial cells were then isolated from PBMCs by incubating with the epithelium-specific antibody BerEP4 conjugated to magnetic beads. Cells were harvested using a magnetic field, lysed, and the protein content was determined and standardized. Telomerase activity was detected in lysates using TeloTAGGG Telomerase PCR ELISA (Roche, Lewes, UK).

Results: Twelve patients who were positive for circulating tumour cells (CTCs) before operation were identified, indicating that the method described may have some value as a diagnostic tool. Sixteen patients were negative for CTCs before surgery but were positive after the operation, indicating that surgical manipulation had resulted in the introduction of tumour cells into the circulation. For 11 of these patients, the CTCs were still detectable at 7 days. The presence of telomerase-positive CTCs did not correlate with Dukes' stage. None of the healthy controls exhibited telomerase activity in epithelial cells.

Conclusion: The method described represents a simple and specific method for the detection of CTCs in patients with colorectal cancer. The detection of telomerase activity in CTCs may have prognostic implications independent of currently established staging systems; the longer-term follow-up of these patients will assess this.

Relaxing properties of L-arginine on the isolated internal anal sphincter are not due to nitric oxide

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Background: Topical L-arginine has been shown to reduce resting anal pressure in healthy human volunteers without significant side-effects and may therefore be of benefit in the treatment of conditions associated with anal hypertonia such as chronic anal fissure. The aim of this *in vitro* study was to investigate whether L-arginine achieves its action via the nitric oxide pathway.

Methods: Using an established sheep internal anal sphincter model, strips of internal sphincter were placed in isolated organ baths. Paired segments were exposed to a cumulative concentration of L-arginine in the presence and absence of (1) 100 μmol l^{-1} N^{G}-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, and (2) 3 μmol l^{-1} 1H-[1,2,4]oxadiazolo[4,3-a]-quinoxalin-1-one (ODQ), a soluble guanylyl cyclase inhibitor. The experiments were repeated with D-arginine.

Results: L-Arginine had little effect on the myogenic tone at concentrations of 1–10 mmol l^{-1}, but resulted in a mean (s.e.m.) reduction of 39·8(3·0) and 78·2(5·0) per cent in baseline tone at 30 and 100 mmol l^{-1} respectively ($n = 17$). The addition of L-NAME ($n = 9$) or ODQ ($n = 8$) did not significantly alter this response. D-Arginine also caused a mean(s.e.m.) reduction in tone of 32·3(3·1) and 65·8(4·6) per cent at 30 and 100 mmol l^{-1} respectively, and again this response was not affected by the addition of L-NAME ($n = 9$).

Conclusion: L-Arginine has a relaxant effect at high concentrations in the sheep anal sphincter model, but it is not stereospecific and is not affected by either L-NAME or ODQ. Therefore, nitric oxide cannot be implicated and another mechanism must be involved.

Adverse effects of *REG* expression on survival in colorectal cancer are independent of *EXTL3* expression

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Background: The *REG1α* gene product induces proliferation in pancreatic epithelial cell lines via its receptor *EXTL3*, and expression of *REG1α* correlates with disease recurrence in patients with colorectal cancer. The aim of this study was to examine the expression of *REG1α*, *REG1β* and the *REG1α* receptor *EXTL3* in colorectal cancer and to establish their relationship to survival.

Methods: Ribonucleic acid was extracted from 57 primary colorectal cancers. *EXTL3* and *REG* gene transcripts were detected by real-time reverse transcription–polymerase chain reaction amplification. Three-year survival data were obtained from patient case notes with approval from the local research ethics committee.

Results: Survival status in relation to gene expression is shown in the *Table*.

Transcript expression	No. surviving at 3 years
*REG1α**	
Negative	14 of 16
Positive	23 of 41
*REG1β**	
Negative	18 of 22
Positive	19 of 35
EXTL3	
Negative	13 of 17
Positive	24 of 40
EXTL3 negative	
REG1α negative	9 of 10
REG1α positive	4 of 7
EXTL3 negative†	
REG1β negative	12 of 13
REG1β positive	1 of 4

*$P < 0·05$ (χ^2 test); †$P = 0·02$ (Fisher's exact test)

Expression levels of *REG1α* were significantly correlated with those of *EXTL3* ($r_S = 0·48$, $P < 0·001$). The adverse effect of *REG* expression on survival was maintained in *EXTL3*-negative tumours, although this was significant only for *REG1β*. Cox regression established *REG1β* as a stronger predictor of death within 3 years of resection than *REG1α* (relative risk 3·79 (95 per cent confidence interval 1·09 to 13·12)).

Conclusion: *REG1α* and *REG1β* expression were significantly associated with a reduced 3-year survival rate following surgical resection for colorectal cancer. The adverse effects of *REG* expression are independent of *EXTL3* expression status.

Effect of irradiation on colorectal tumour cell invasion: role of matrix metalloproteinases and integrins

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Background: Matrix metalloproteinase (MMP) expression is increased after preoperative radiotherapy but there is no evidence that this results in increased cell invasion. This study addressed the effect of irradiation on the invasion of a colorectal tumour model in a functionality assay.

Methods: Colorectal fibroblasts (ccd-18Co) were co-cultured with an increasing ratio (0·001–2 : 1) of the human colorectal tumour cell line LoVo. MMP expression and activation was measured by zymography. The fibroblast : epithelial cell ratio resulting in maximal MMP expression was used to study invasion on the mock basement membrane, Matrigel. $\alpha V\beta 3$ integrin gene expression was measured by real-time polymerase chain reaction as it plays a role in MMP-2 activation.

Results: Maximal expression of pro-MMP-2 protein was seen at a ratio of 5 : 1 tumour cells : fibroblasts (1·2-fold increase; $P = 0{\cdot}01$, Student's *t* test). This was accompanied by a 1·7-fold increase in gene expression of the αV subunit ($P = 0{\cdot}05$, Student's *t* test). The level of the $\beta 3$ subunit was not increased. On Matrigel, greater numbers of cells invaded 48 h after irradiation with 4 Gy than with controls (2·7-fold increase; $P = 0{\cdot}08$, one-way analysis of variance), although absolute cell numbers were decreased after irradiation.

Conclusion: In this model irradiation appears to select tumour cells that are more invasive, possibly as a result of increased MMP-2 expression and activation. Selective inhibition of MMP expression and activation may lower recurrence rates further after adjuvant radiotherapy.

Hand-assisted laparoscopic surgery improves task performance compared with the conventional laparoscopic approach

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Background: Hand-assisted laparoscopic surgery (HALS) has been introduced to overcome the restrictions of minimal access surgery while maintaining its benefits. The aim of this study was to compare task performance using hand-assisted laparoscopic techniques compared with the conventional laparoscopic approach.

Methods: The standard task entailed closure of a 3-cm enterotomy constructed from a non-live porcine small bowel inside a laparoscopic training simulator designed for HALS. A hand access device was used to introduce the internal hand to the operative field. Ten surgeons with no previous laparoscopic experience participated in the study. Surgeons received similar training in HALS and the conventional approach. Each surgeon sutured three times using HALS and conventional laparoscopy in a random sequence. The endpoints were the execution time (min), intraluminal bowel pressure causing water leakage through the suture line (cmH_2O) and suture placement error score (deviation (in millimetres) of the entry and exit points less than 3 mm or more than 5 mm from the enterotomy edge and apart). Statistical analysis was with the *t* test for independent samples; $P < 0{\cdot}05$ was considered significant.

Results: The execution time was shorter for HALS (mean(s.d.) 15·1(0·9) min) than for conventional laparoscopy (22·1(1·6) min) ($P < 0{\cdot}001$). HALS also resulted in a significantly higher mean(s.d.) leakage pressure (67·0(2·6) *versus* 52·2(3·6) cmH_2O) and a lower suture placement error score (1·5(0·3) *versus* 6·8(1·1) mm) compared with conventional laparoscopy ($P < 0{\cdot}005$).

Conclusion: The quality of bowel suturing was higher using a hand-assisted laparoscopic technique compared with conventional laparoscopy.

Dendritic cells are redistributed from the circulation into the colonic mucosa in patients with colorectal cancer

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Background: Increased infiltration of antigen-presenting dendritic cells (DCs) in colorectal cancer (CRC) is associated with prolonged survival. It was hypothesized that these tissue-infiltrating DCs are redistributed from the circulation; the levels of DCs in the blood, CRC, adjacent bowel and mesenteric lymph nodes were measured.

Methods: Flow cytometric analyses were performed on preoperative peripheral venous blood samples. DCs were defined as negative for surface expression of CD3, CD14, CD16, CD19, CD20 and CD56, and as positive for human leucocyte antigen (HLA)-DR. Immunohistochemical staining for immature DCs (Langerhans cells expressing CD1a and Langerin) and for mature DCs expressing DC-LAMP was carried out on resected colorectal cancer, adjacent non-cancerous bowel and mesenteric lymph nodes.

Results: Seventy-eight patients with colorectal cancer and 61 'no-cancer' controls were studied. The level of circulating DCs in patients with colorectal cancer (median 0·25 per cent) was significantly reduced compared with that in controls (0·41 per cent; $P < 0{\cdot}001$). No increase in the absolute counts of lymphocytes, neutrophils and monocytes was found, indicating a true reduction in circulating DCs. No correlation was found between circulating DCs and mature DCs infiltrating in the cancer. Significantly more mature DCs were found in cancer and mesenteric lymph nodes than in adjacent non-cancerous bowel from patients with colorectal cancer. There was a negative relationship between circulating DCs and immature DC infiltration (CD1a-positive: Kruskal–Wallis statistic 20·9, $P < 0{\cdot}001$; Langerin-positive cells: Kruskal–Wallis statistic 21·0, $P < 0{\cdot}001$) in the non-cancerous mucosa from patients with cancer but not in the tumour or lymph node.

Conclusion: These clinical findings suggest that a cancer-related stimulus causes immature DCs to be taken up from the blood by mucosa adjacent to colorectal cancer. DCs mature in the colorectal cancer but not in adjacent mucosa, and migrate to the lymph nodes.

Physiological changes of the anorectum following pelvic radiotherapy

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Background: Faecal frequency, urgency and incontinence are common following pelvic radiotherapy, but the precise aetiology of these symptoms remains unclear. The aim of this study was to evaluate prospectively the effects of pelvic radiotherapy on anorectal function.

Methods: Thirty-one men with carcinoma of the prostate (28 patients) and bladder (three) completed incontinence/proctitis symptom scores and anorectal physiological studies before and 6 weeks after pelvic radiotherapy; at 6 months 19 of the patients were studied. The results were compared with the Wilcoxon signed rank test.

Results: At 6 weeks, median incontinence (0 *versus* 4; $P < 0{\cdot}001$) and proctitis (0 *versus* 2; $P < 0{\cdot}001$) scores altered significantly. The median anal canal resting (83 *versus* 79 cmH$_2$O; $P < 0{\cdot}14$) and squeeze (152 *versus* 162 cmH$_2$O; $P < 0{\cdot}27$) pressures were unaltered. The median maximum tolerable rectal volume (245 *versus* 194 ml; $P < 0{\cdot}005$) and median rectal electrical sensitivity (20 *versus* 22 mV; $P < 0{\cdot}04$) altered significantly. After 6 months, compared with preradiotherapy values, the median incontinence (0 *versus* 1; $P < 0{\cdot}02$) and proctitis (0 *versus* 2; $P < 0{\cdot}005$) scores were significantly altered, as were anal canal resting pressure (83 *versus* 73 cmH$_2$O; $P < 0{\cdot}001$) and median maximum rectal volume (245 *versus* 194 ml; $P < 0{\cdot}005$).

Conclusion: Acute anorectal symptoms associated with proctitis 6 weeks after pelvic radiotherapy are related to an increased rectal sensation and diminished maximum rectal tolerated volume. Symptoms at 6 months could be explained by the decrease in anal canal resting pressure and diminished maximum rectal tolerated volume.

Heparin attenuates ischaemia–reperfusion-induced leucocyte–endothelium interactions in the colonic microvasculature

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Background: Induction of ischaemia–reperfusion (IR) causes increased leucocyte rolling, adhesion and extravasation. Heparin can neutralize products of the complement cascade and inhibit chemoattractant-induced leucocyte adhesion *in vitro* and tissue accumulation of leucocytes *in vivo*, potentially through binding to CD11b/CD18. However, it is not known whether heparin may interfere with leucocyte–endothelium interactions in the colon.

Methods: The superior mesenteric artery was occluded for 30 min in male C57/Bl6 mice. Leucocyte rolling and firm adhesion in colonic postcapillary venules were analysed by means of an inverted intravital microscope after 120 min of reperfusion. Heparin (50–5000 units kg^{-1}) was given immediately before reperfusion.

Results: In untreated animals, mean(s.d.) leucocyte rolling and adhesion were 29(3) cells per min and 79(25) cells per mm respectively. Notably heparin at 5000 units kg^{-1} significantly reduced leucocyte rolling and adhesion by 89 and 80 per cent respectively. Heparin at doses of 500 and 50 units kg^{-1} had no effect on leucocyte responses in the colon.

Conclusion: Heparin reduces leucocyte rolling and adhesion in a dose-dependent manner. These results may help to explain a protective effect of heparin in IR-induced tissue injury.

Assessment of outcome from colorectal carcinoma using an artificial neural network

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Background: Accurate and consistent outcome prediction is essential in the management of patients with colorectal carcinoma. The aim of this study was to assess the ability of an artificial neural network (ANN) to predict outcome from colorectal carcinoma, thereby individualizing the decision-making process in terms of follow-up and adjuvant therapy.

Methods: Consecutive patients who presented with Dukes' A–C colorectal carcinoma from January 1992 to December 1999 were identified from the St Vincent's Hospital colorectal cancer registry. Prospectively collected American Joint Committee on Cancer category prognostic markers were included in the analysis. Two- and five-year follow-up data were collected for these patients; censored data were excluded. A multilayered perceptron ANN was constructed and trained using the data. Twenty-five per cent of data sets were excluded from training and were used to evaluate the accuracy of predicting 5-year disease-free survival in individual patients using the trained ANN. The accuracy of prediction was analysed using a confusion matrix and compared with multivariate logistic regression analysis.

Results: Some 1064 patients presented with colorectal carcinoma, of whom 801 were available for analysis. Of these patients 157 had Dukes' A, 353 had Dukes' B and 291 had Dukes' C tumours on presentation. Disease-free survival rates at 2 and 5 years were 79 and 58 per cent respectively. The mean squared error (MSE) of the ANN at predicting disease-free survival was 0·04 (accuracy 92 per cent). This was significantly better than with the logistic regression model (MSE 0·14, accuracy 77 per cent; $P < 0{\cdot}01$).

Conclusion: ANNs are able to predict outcome from colorectal carcinoma in individual patients with accuracy and outperform regression analysis. ANN is a valuable tool in management of patients with colorectal carcinoma.

Quantification of lymphangiogenesis in breast cancer using a lymphatic endothelial marker, LYVE-1

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Background: Lymphangiogenesis plays an important part in cancer growth and metastasis. Research into lymphangiogenesis has been limited by the lack of specific markers. Using a novel, highly specific, lymphatic endothelial marker, LYVE-1, lymphangiogenesis in lymph node-positive breast cancers, node-negative tumours and background normal breast tissue was quantified.

Methods: RNA was extracted and equal concentrations of complementary DNA (cDNA) were synthesized from 103 blinded archival breast specimens. Following conventional polymerase chain reaction (PCR) with LYVE-1 primers, the PCR product was sequenced and found to be homologous with the LYVE-1 DNA sequence. The LYVE-1 PCR product was subsequently cloned, and plasmids containing the sequence were extracted and used as internal standards. Real-time quantitative PCRs were performed on the breast cDNA and plasmids, using a Scorpion detection system with primers specific for a smaller part of the LYVE sequence. Reactions were repeated four times to minimize errors. The original concentration of LYVE-1 cDNA was deduced by reference to the plasmid standards. Results between the nodal groups were compared with Student's t test.

Results: LYVE-1 messenger RNA was found in 98 of 103 specimens. The mean concentrations of RNA in the specimens (copy numbers per microlitre) were as follows: node-positive tumours 2853·8 ($n=32$); node-negative tumours 369·9 ($n=58$); background breast tissue 12·5 ($n=13$). The difference between node-positive and node-negative tumours was significant ($P=0{\cdot}04$).

Conclusion: It is possible to quantify lymphangiogenesis by using a highly specific lymphatic marker. With this technique the rate of lymphangiogenesis was found to be higher in node-positive breast cancers than in node-negative tumours.

Cyclo-oxygenase 2 and growth factor receptor expression in breast neoplasia

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Background: Cyclo-oxygenase (COX) type 2 is overexpressed in colorectal cancer, and COX inhibition prevents the development of colonic cancer. COX-2 is also overexpressed in breast cancer, although the mechanism of COX-2 upregulation in neoplasia is not clear. This study aimed to determine whether COX-2 expression is related to the expression of hormone or growth factor receptors, tumour grade or cellular proliferation in ductal carcinoma *in situ* (DCIS) or invasive breast cancer (IBC).

Methods: COX-2, C-erbB2, epidermal growth factor receptor (EGFR), oestrogen receptor (ER) and Ki67 receptor expression were determined by immunohistochemistry of paraffin sections and assessed by light microscopy. Expression was scored from 0 to 4 for each marker.

Results: COX-2 expression in DCIS ($P<0{\cdot}001$) and IBC ($P<0{\cdot}001$) was significantly greater than in normal breast. There was no difference in COX-2 expression level between DCIS and IBC ($P=0{\cdot}87$). In DCIS, COX-2 expression correlated significantly with C-erbB2 expression ($P=0{\cdot}05$), but not with that of EGFR, ER, Ki67 or tumour grade. In invasive cancer, COX-2 correlated significantly with EGFR ($P=0{\cdot}02$) but with no other parameter.

		COX-2 staining index					
Tissue	n	0	1	2	3	4	P
Normal breast (breast reduction)	36	21	12	3	0	0	
Normal breast around DCIS	46	33	5	7	1	0	0·44
Invasive cancer	54	6	12	12	21	3	< 0·001
DCIS	96	10	14	35	29	8	< 0·001

Conclusion: The association of COX-2 expression with growth factor expression in DCIS and IBC suggests that growth factor receptor activation is involved in COX-2 upregulation in breast neoplasia. COX-2 inhibition may have a role in EGFR and C-erbB2-positive breast cancer.

Intraoperative assessment by optical biopsy for lymph node metastasis in breast cancer

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Background: Intraoperative assessment of the sentinel lymph node (SLN) enables the surgeon to decide on immediate axillary lymph node dissection at the time of SLN biopsy. The aim of this study was to develop a device (optical biopsy system; OBS) for use during surgery that is capable of assessing lymph nodes (LNs) for metastasis in patients with invasive breast cancer, using the principles of elastic scattering spectroscopy (ESS).

Methods: ESS involves the spectral analysis of light scattering by intracellular and extracellular components, and is sensitive to morphological changes in tissue. The optical biopsy process involves the delivery of light from a light source directly on to the sectioned surface of LNs via an optical fibre. After scattering by the tissue, the light is collected by a second optical fibre and analysed by a spectrometer and a computer, generating an optical spectrum. The optical spectrum is analysed against a training set of optical spectra from LNs using model-based analysis to determine the status of the LN. The result was compared with the histological findings.

Results: In total, 75 LNs were tested. OBS provided a diagnosis in 72 LNs (three nodes were deemed indeterminate) and was correct in 62 nodes. OBS was able to identify metastasis correctly in 16 of 19 LNs, compared with histological findings. There were seven false positives and three false negatives, giving a sensitivity of 84·2 per cent and a specificity of 86·8 per cent.

Conclusion: Current intraoperative techniques to assess SLNs are imprint cytology and fresh-frozen section, which are time consuming and operator dependent. The OBS has the potential to provide instant and non-operator-dependent intraoperative analysis of SLNs in patients with breast cancer.

Analysis of potential risk factors for failed localization of axillary sentinel nodes in breast cancer

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Background: Sentinel node biopsy is increasingly used in the management of breast cancer. The aim of this study was to determine factors that may lead to an increased incidence of failed sentinel node localizations in patients with breast cancer.

Methods: The data for 500 patients from the audit phase of the UK ALMANAC trial were analysed. The following variables were studied: patient body mass index (BMI), site of primary tumour (upper outer or inner, lower outer or inner quadrants), histopathological size and type of tumour, and axillary nodal status (positive or negative). Results were compared with Student's t test and the χ^2 test.

Results: The mean(s.d.) BMI for failed and successful sentinel node localizations was 32·96(7·39) and 25·91(4·44) respectively ($P < 0.001$). The incidence of failed localizations in tumours in the different breast quadrants was as follows: 2·1 per cent (upper outer), 2·2 per cent (lower outer), 7·0 per cent (upper inner) and 11·1 per cent (lower inner) ($P < 0.001$). Failed localizations occurred in 3·1 per cent of tumours up to 20 mm in size and in 4·2 per cent tumours over 20 mm. Some 8·9 per cent of invasive lobular carcinomas were failed localizations, compared with 2·1 per cent of invasive ductal carcinomas and no tubular or mucinous tumours. Finally, failed localizations occurred in 3·7 per cent of node-positive axillas and in 1·8 per cent of node-negative axillas.

Conclusion: A high BMI (over 30), tumours in the inner half of the breast and invasive lobular carcinomas were found to be statistically significant risk factors for failed sentinel node localization in breast cancer. In contrast, tumour size and node status did not appear to affect localization.

Hypoxia induces augmented malignant potential via nuclear factor κB-mediated metalloprotease upregulation in a variety of adenocarcinomas

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Background: Hypoxia within solid adenocarcinomas and protease upregulation have been independently implicated as poor prognostic indicators in a variety of tumour types. The authors hypothesized that matrix metalloproteases (MMPs), lacking a hypoxic response element, are upregulated via a nuclear factor (NF) κB-dependent pathway.

Methods: Colonic (SW1222), breast (MDA-MB231) and pancreatic (PSN-1) tumour cell lines were exposed to hypoxia (1 per cent oxygen, 94 per cent nitrogen, 5 per cent carbon dioxide) for a period of 24 h. Invasion across Matrigel™-coated 8-μm Transwell™ filters as well as cell viability was assessed using a non-radioactive cell proliferation assay (Promega, Southampton, UK). Activity of MMP-2 and MMP-9 was assessed by means of gelatin zymography. Expression of tissue inhibitor of metalloproteases (TIMP) 1 was quantified using an enzyme-linked immunosorbent assay (Biotrak; Amersham Pharmacia Biotech, UK). Inhibition of invasion assays was performed using a specific gelatinase inhibitor (MMPI; Calbiochem, Nottingham, UK). NF-κB inhibition of zymography was performed using SN50 (Biomol, UK). Electromobility band shift assays were utilized to characterize the NF-κB response to hypoxia, together with Western blotting for its inhibitor, I-κB. Data were analysed with the Mann–Whitney U and Kruskal–Wallis tests.

Results: All tumour lines demonstrated augmented invasion over 72 h ($P < 0.01$ for all groups). A concomitant significant increase in MMP-2 and MMP-9 activity was observed in the SW1222 and PSN-1 lines, and MMP-9 and 103-kDa gelatinase in MDA-MB231 ($P < 0.001$). This increased activity was attenuated by the addition of SN50 ($P < 0.001$). TIMP-1 expression was significantly lower in the hypoxic group ($P < 0.05$). NF-κB demonstrated a band shift in response to hypoxia, with levels of I-κB significantly decreased on Western blotting ($P < 0.001$).

Conclusion: Hypoxia induces an increased invasive capacity via gelatinase upregulation mediated by NF-κB without loss in cell viability. This provides a novel mechanism explaining the poorer prognosis seen in patients with protease-secreting solid adenocarcinomas.

Does stopping hormone replacement therapy in newly diagnosed breast cancer reduce epithelial proliferation?

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Background: Stopping hormone replacement therapy (HRT) before surgery leads to increased vasomotor disturbances but may potentially prevent tumour growth. This study aimed to determine the effect of stopping HRT on epithelial proliferation and its relation to receptor status and grade in early breast cancer.

Methods: A study was undertaken of 85 women who, at diagnosis by Tru-cut (Travenol Laboratories, Thetford, UK) biopsy, were on HRT (70 stopped HRT and 15 controls continued taking HRT until operation). Oestrogen receptor (ER), progesterone receptor (PR) and Ki67 labelling index (a measure of epithelial cell proliferation) were determined by immunohistochemistry on paired sections of the core biopsy and operative specimens for each patient. Tumours with more than 5 per cent cell nuclear staining were considered ER and PR positive.

Results: Women who stopped HRT showed a significant drop in proliferation compared with those who continued HRT ($P = 0{\cdot}02$).

HRT group	ER*	PR*	Median core Ki67†	Operative Ki67†	P
Stopped	+ (56)	+ (48)	7·0 (2·6–9·2)	5·2 (2·3–6·5)	0·002
Stopped	− (14)	− (22)	16·6 (5·3–23·7)	15·3 (4·8–21·8)	0·52
Continued	+ (12) − (3)	+ (12) − (3)	2·7 (1·8–3·3)	3·6 (2·1–4·7)	0·20

Values in parentheses are *number of patients and †interquartile range

The fall in proliferation was seen in ER- and PR-positive tumours and was significantly greater in grade 1 ($n = 45$) than in grade 2 and 3 ($n = 25$) tumours ($P = 0{\cdot}01$).

Conclusion: Withdrawal of HRT in patients with newly diagnosed breast cancer is necessary for ER-positive, PR-positive tumours. There was greater benefit in stopping HRT in lower-grade than in higher-grade tumours.

Vascular endothelial growth factor blockade increases tumour cell apoptosis under hypoxic conditions

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Background: Vascular endothelial growth factor (VEGF) is a survival factor for tumour cells, and hypoxia increases the release of VEGF. It was hypothesized that VEGF acts as a survival factor for tumour cells, protecting them from apoptosis under hypoxic conditions

Methods: Murine (4T1) and human (MDA-MB-231) mammary adenocarcinoma cells were incubated with neutralizing antibody to VEGF ($1\ \mu g\ ml^{-1}$) or recombinant VEGF ($100\ ng\ ml^{-1}$) under normoxic and hypoxic conditions. VEGF and Bcl-2 expression were assessed by Western blotting, and tumour cell apoptosis was determined with the TUNEL (terminal deoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate nick end labelling) assay.

Results: Hypoxia significantly increased VEGF expression by both tumour cell lines (4T1: normoxia 0·15 *versus* hypoxia 0·36 pg per μg protein; MDA-MB-231: normoxia 7·35 *versus* hypoxia 12·18 pg per μg protein; $P < 0{\cdot}05$). Neutralizing antibody to VEGF blocked hypoxia-induced expression of Bcl-2. Anti-VEGF antibodies induced tumour cell apoptosis under hypoxia (4T1: hypoxia 6 per cent *versus* hypoxia plus anti-VEGF 13 per cent; MDA-MB-231: hypoxia 7·2 per cent *versus* hypoxia plus anti-VEGF 20 per cent).

Conclusion: In addition to its angiogenic activity, this study confirmed that VEGF prevents tumour cell apoptosis by increasing Bcl-2 expression. Anti-VEGF strategies may sensitize hypoxic and therefore resistant tumour cells to chemotherapy and irradiation.

Epidermal growth factor receptor control of epithelial proliferation and progesterone receptor expression in normal breast epithelium

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Background: Oestrogen is the major hormone controlling the proliferation and induction of the progesterone receptor (PR) in normal breast epithelium, but primary culture of breast produces oestrogen receptor (ER)-negative cells which proliferate in response to epidermal growth factor (EGF) stimulation.

Methods: To determine the role of EGF receptor (EGFR) in controlling the proliferation and expression of PR in the normal breast, a study was undertaken of human breast tissue implanted into athymic nude mice from 19 premenopausal women. After 14 days mice received either control gavage, Iressa (an EGFR tyrosine kinase inhibitor; TKI) gavage, oestradiol 2-mg (E2) implant plus control gavage, or oestradiol and Iressa gavage. Implants were removed on days 14, 21 and 28, and proliferation was determined by means of Ki67 immunohistochemical labelling and PR expression.

Results: EGFR TKI inhibited both unstimulated and oestrogen-stimulated epithelial proliferation.

Group	Ki67 LI at day 28	PR at day 14
Control gavage	3·9 (2·3, 6·0)	13·9 (12·3, 15·6)
Iressa gavage	2·0 (1·4, 3·3)*	10·6 (8·9, 12·9)*
Control gavage + E2 implant	4·9 (4·3, 5·9)*	26·0 (23·4, 29·9)*
Iressa gavage + E2 implant	1·5 (1·0, 2·2)†	20·7 (18·3, 23·6)†

Values are percentage (95 per cent confidence interval) $^*P < 0{\cdot}001$ *versus* control gavage; $^\dagger P < 0{\cdot}001$ *versus* control gavage plus E2 implant

Conclusion: The EGFR is central in control of growth and induction of oestrogen-induced gene expression in normal breast. Blockade of the EGFR may represent a novel chemoprevention strategy for breast cancer.

Probiotics confer protection against perioperative metastatic tumour growth

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Background: Bacterial translocation and endotoxaemia enhance perioperative metastatic growth. As probiotics alter the enteric microflora, their effect on metastatic development after surgery was investigated.

Methods: Balb/c mice ($n = 40$) underwent a tail vein injection of 1×10^5 highly metastatic murine adenocarcinoma 4T1 cells. Animals were fed control or probiotic feed containing *Lactobacillus salivarius* (subspecies *salivarius*) and *Bifidobacterium infantis* up to and following laparotomy on day 14. All were killed after 28 days, when serum endotoxin was assayed using the *Limulus* amoebocyte assay. Metastatic burden was evaluated using lung : body-weight ratio and a metastatic index (mean number of metastases per ten low-power fields). Mitotic counts and TUNEL (terminal deoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate nick end labelling) apoptotic indices (mean number of apoptotic cells per metastasis) were established.

Results: In animals receiving probiotic feed, surgically induced increments in metastatic tumour growth were abolished. This correlated with a significant reduction in mitotic count, metastatic load and serum endotoxin levels.

Animal group ($n = 10$ per group)	Apoptotic index	Mitotic count	Metastatic index	Lung : body-weight ratio	Serum LPS (units ml^{-1})
No probiotics					
No surgery	5·2(0·4)	67(3·1)*	6·5(0·3)*	1·5(0·4)*	0·8(0·1)*
Surgery	2·6(0·1)*	62·4(8·3)	11·0(0·5)	2·9(0·1)	1·6(0·1)
Probiotics					
No surgery	4·0(0·1)	36·8(6·4)*	4·0(0·3)*	1·6(0·1)*	0·9(0·1)*
Surgery	2·4(0·1)*	44·0(5·6)*	5·4(1·3)*	1·5(0·1)*	0·9(0·1)*

Values are mean(s.e.m.). LPS, lipopolysaccharide. *$P < 0.05$ (analysis of variance)

Conclusion: These results endorse a role for the gut in postoperative metastatic tumour growth. They identify a novel strategy for protection against metastatic tumour growth in the perioperative period.

Novel stool-based screening test for colorectal cancer using antibodies directed against DNA replication protein, minichromosome maintenance protein 2

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Background: The aim was to develop a stool-based screening test for colorectal cancer (CRC) using antibodies directed against minichromosome maintenance protein 2 (Mcm2). Mcm2 is a member of the preinitiation complex essential for DNA replication and is an accurate indicator of cell cycle entry by neoplastic cells.

Methods: Using HT-29 CRC cells applied to the surface of normal stool and exposed to different conditions, a protocol was developed for optimal CRC cell retrieval and detection of Mcm2. Sixty subjects were then recruited into a clinical evaluation study with ethics committee approval. Colonocytes were retrieved from the stool surface using three techniques: (1) direct smear, (2) centrifugation of a suspended solution washed over the stool surface and (3) addition of magnetically labelled microbeads. Expression of Mcm2 by colonocytes was assessed by means of immunocytochemistry.

Results: The HT-29 model showed that CRC cell yield is adequate for up to 8 h after defaecation, with an improved yield on ice. More than 95 per cent of HT-29 CRC cells express Mcm2 up to 12 h after defaecation. In all 20 normal volunteer subjects, colonocytes were retrieved but showed no expression of Mcm2. Of 40 patients with CRC (32 left-sided and eight right-sided), cells were retrieved from 36. In all of these cases, Mcm2 was expressed by the CRC cells. The direct smear and centrifugation techniques enabled retrieval of Mcm2-positive cells in significantly more cases than the magnetic microbeads ($P < 0.001$ for each). A combination of smear and centrifugation techniques was adequate for isolation of CRC cells in 35 of the 36 test-positive cases. To date, the performance of the test overall is: sensitivity 90 per cent, specificity 100 per cent, positive predictive value 100 per cent and negative predictive value 83 per cent.

Conclusion: This new technique shows that a high percentage of colorectal cancers can be detected by immunocytochemical analysis of exfoliated cells from the surface of human stool.

Fluorescence imaging endoscope for early detection of gastrointestinal malignancy

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Background: A fluorescence imaging endoscope that utilizes 5-aminolaevulinic acid (ALA), an exogenous photosensitizer, has been developed for the early detection of gastrointestinal cancer. The first prototype displays the fluorescence and white light images separately, while the second prototype is equipped with computer software that allows the two images to be superimposed, thus enabling biopsies to be taken from suspicious areas under direct vision.

Methods: After obtaining ethical approval, 25 patients with histologically proven gastrointestinal cancer were sensitized with 5-aminolaevulinic acid (ALA) 20–40 mg kg^{-1} orally 4–6 h before endoscopy. Twenty patients (ten with colorectal, five with oesophageal and five with gastric cancer) were tested with the first prototype and five patients (all with rectal tumours) with the second prototype.

Results: Fluorescence endoscopy using both prototypes positively identified all tumours (sensitivity 100 per cent). However, there was a high false-positive rate (specificity 70 per cent). As the two images in the first prototype are separate, accurate localization of tumours in the navigational image was difficult. The second prototype generated a composite image that accurately localized suspicious areas on the colour image, thus enabling biopsies to be taken for further evaluation.

Conclusion: This fluorescence detection system is highly sensitive for the detection of gastrointestinal tumours. Superimposing fluorescent images on white light images is an important development, and allows accurate localization of suspicious areas. Further work is being carried out to improve the specificity of the system.

Mortality and stage distribution amongst patients undergoing gastrectomy for cancer: initial results of a multicentre prospective cohort study

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Background: The optimal surgical management of gastric cancer remains unclear following trials of D_2 nodal dissection. An attempt was made to delineate current UK surgical practice and outcomes, particularly the associations of case volume, nodal dissection (D_1 or D_2) and patient fitness with morbidity and mortality.

Methods: A multicentre prospective cohort study of patients with gastro-oesophageal cancer in 30 Trusts in England and Wales (ASCOT) began on 1 January 1999. The morbidity and mortality results for patients with gastric cancer amongst the first 1000 cases entered are presented.

Results: Some 52 per cent of patients had gastric cancer, 24 per cent had junctional and 24 per cent had oesophageal cancer. Most gastric cancers were locally advanced and there was significant co-morbid pathology. No significant difference was seen between high- and low-volume units, or between level D_1 and D_2 gastrectomy (see *Table*). The *Table* also shows the percentage of cases in each tumour (T) stage, node (N) stage and American Society of Anesthesiologists (ASA) grade.

	Percentage
Tumour stage	
T_1	19
T_2	25
T_3	46
T_4	10
Node stage	
N_0	35
N_1	48
N_2	12
$> N_2$	5
ASA grade	
I	19
II	51
III	28
IV	2
Mortality rate	
D_1 resection	8·5
D_2 resection	7·4
< 50 per year*	10·4
> 50 per year*	8·1

*Number of cases reported by unit per year

Mean postoperative mortality was 16 per cent for total and 6 per cent for subtotal gastrectomy.

Conclusion: Postoperative mortality following gastrectomy for cancer in England remains high, but no effects of case volume or type of nodal dissection were observed. Most patients present with advanced disease and significant co-morbid pathology.

Physiological effects of exercise training for intermittent claudication: results of a randomized controlled trial

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Background: The physiological mechanisms underlying symptomatic benefits of exercise training for intermittent claudication remain unclear. The aim of this study was to investigate the functional and physiological effects of supervised exercise.

Methods: Patients with stable symptoms of intermittent claudication were randomized to either 3 months of supervised exercise or a control group receiving exercise advice alone. Patients were assessed before randomization and again after 3 and 6 months. Measurements included treadmill walking distance, ankle : brachial pressure index

(ABPI), heart rate, blood pressure and serum lactate responses to exercise. Statistical analysis was with the Mann–Whitney U test.

Results: Sixty patients were randomized; 53 patients were followed successfully for 3 months. Supervised exercise increased both pain-free walking distance (PFWD) and maximal walking distance (MWD) compared with controls at 3 months (median percentage change for PFWD: 87·1 *versus* 24·0 per cent, P = 0·004; MWD: 92·3 *versus* 25·2 per cent, P = 0·09), with improvements persisting at 6 months. At follow-up, patients in the supervised group had a significant reduction in resting arterial pressure compared with controls (mean (interquartile range) −8·1 (−15·5 to 1·6) *versus* −1·6 (−12·7 to 6·2) mmHg, P = 0·04). Serum lactate concentration at maximal claudication was significantly greater at 3 months in the supervised exercise group compared with controls (0·87 (0·17 to 1·66) *versus* −0·14 (−0·48 to 0·50) mmol l^{-1}, P = 0·008). This difference declined by 6 months. Resting heart rate, heart rate at maximum claudication and ABPI measurements remained unchanged.

Conclusion: Supervised exercise significantly improved claudication distance. These improvements were accompanied by a reduction in resting blood pressure and an increase in serum lactate concentration at maximum claudication, which subsequently declined at 6 months despite continued improvement in walking distance.

Pharmacological preconditioning attenuates lower-torso ischaemia–reperfusion-induced diaphragmatic dysfunction

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Background: Respiratory complications occur in up to 60 per cent of patients after aortic surgery. Teprenone is a known heat shock protein inducer licensed for use in humans.

Methods: The aim of this study was to determine the effect of Teprenone on diaphragmatic dysfunction secondary to lower-torso ischaemia–reperfusion (IR) injury. Male Sprague–Dawley rats (n = 36) were randomized into four groups: control, IR, IR plus vehicle, and IR plus Teprenone, which was given by gavage 24 h before IR. IR was achieved by placing an infrarenal aortic clamp for 60 min followed by 24 h of reperfusion. Diaphragm muscle contractile function was assessed using electric field stimulation in a tissue bath. Heat shock protein expression was evaluated by Western blotting.

Results:

	Control	IR	Teprenone
Twitch (g)	197(17)*	119(12)	168(14)*
Tetanus (g)	312(25)	286(28)	291(24)
Fatiguability (s)	29·0(2·4)	23·5(1·6)	31·7(1·4)*

Values are mean(s.e.m.). *P < 0·05 *versus* IR (analysis of variance)

Conclusion: Teprenone attenuates aspects of diaphragmatic function after lower-torso IR injury, especially fast fibres and fatiguability. This clinically licensed drug should be evaluated for use in vascular reconstructive surgery.

Disease profile of complete pathological responders following neoadjuvant chemoradiotherapy and curative resection in oesophageal cancer

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Background: The use of neoadjuvant chemotherapy in the treatment of oesophageal cancer results in approximately 25 per cent of patients exhibiting a complete response at histological examination. This study examined the overall survival, disease-free survival, disease recurrence and patterns of first failure for complete pathological responders compared with patients with stages I, IIa (node negative) and IIb–III (node positive) disease after chemotherapy and surgical resection.

Methods: A total of 69 patients treated with neoadjuvant chemotherapy and R_0 surgical resection were followed prospectively over a 10-year period with a median follow-up of 40 months. Twenty-one patients had a complete pathological response (CPR) while 14, 18 and 16 patients were staged histologically at stages I, IIa and IIb–III respectively.

Results: The complete responders did not have a significantly higher median overall survival compared with patients with stages I and IIa (55 *versus* 44 *versus* 46 months respectively; P = 0·91, log rank test). The CPR group did have a significantly higher median survival than patients with stage IIb–III disease (55 *versus* 16 months; P = 0·04). Failure was predominantly systemic rather than local in the CPR group. Univariate and multivariate analysis demonstrated that nodal status, squamous cell histology and worsening tumour cell differentiation were associated with disease recurrence.

Conclusion: Following neoadjuvant chemotherapy and R_0 surgical resection, patients with stage I and IIa disease have a similar survival to patients who exhibit a CPR. Nodal status remains the critical predictor of outcome in oesophageal cancer.

Interleukin 12 polymorphisms in a benign dyspeptic population

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Background: Cytokine function is believed to play a role in benign and malignant mucosal disease. Polymorphisms at host genetic level may be important in determining susceptibility to different mucosal responses. The aim of this study was to examine the polymorphisms of interleukin (IL) 12, a proinflammatory cytokine, in a large benign dyspeptic group with a high prevalence of *Helicobacter pylori* infection.

Methods: The study cohort, a dyspeptic population ($n = 300$) on acid suppression therapy, was subdivided into ulcer ($n = 96$), non-ulcer ($n = 156$) and 'not investigated' ($n = 48$) categories. DNA was extracted from peripheral blood leucocytes and the distribution of the IL-12 alleles was determined by polymerized chain reaction, followed by restriction enzyme digestion.

Results: The genotype frequencies for the study population were: A/A, 66·3 per cent; A/C, 29·7 per cent; and C/C, 4 per cent. These are similar to the normal control frequencies of 64·2, 31·6 and 4·2 per cent respectively. However, the genotype frequencies for patients with ulcer (42) *versus* non-ulcer (56) in the *Helicobacter*-positive group were 59·5, 38·1 and 2·4 per cent *versus* 78·6, 16·1 and 5·4 per cent respectively ($P = 0{\cdot}04$).

Conclusion: There is a significant difference between patients with a *Helicobacter*-positive ulcer and those without an ulcer. The authors have previously demonstrated a similar increased frequency of the A/A genotype in patients with *H. pylori*-positive gastro-oesophageal cancer, and this study therefore suggests that the polymorphisms of the proinflammatory cytokine IL-12 influence the host response to *H. pylori* infection.

Proliferation and apoptotic gene expression in oesophageal cancer: predictors of response to neoadjuvant chemotherapy and radiotherapy

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Background: Cancer cell proliferation and altered expression of the genes regulating apoptosis (survivin, bcl-2 and p53) may modulate tumour sensitivity to cytotoxic agents and act as molecular markers of response to treatment.

Methods: Forty patients who had received preoperative chemoradiotherapy (45 Gy plus fluorouracil–cisplatin; $n = 32$) or chemotherapy alone (epirubicin–cisplatin–5-fluorouracil; $n = 8$) were studied. Pretreatment biopsies were immunostained with antisurvivin, p53, bcl-2 and Ki-67 antibodies using a standard technique. Survivin immunostaining was scored by the percentage and intensity of cancer cells immunostained, and expression of survivin was classfied as high or low. Bcl-2 and p53 was scored as positive if more than 10 per cent of cells were immunostained. A proliferative index was calculated by counting the number of Ki-67-immunostained nuclei in more than 1000 cancer cells. Tumour response to treatment was evaluated as a complete response (CR), a partial response (PR) or no response (NR) depending on histopathological examination of the resected specimen and by radiological imaging following treatment.

Results: Complete responders had tumours with significantly greater proliferative indices compared to partial and non-responders. Survivin, bcl-2 and p53 did not influence the response of the tumours.

	CR ($n = 9$)	PR ($n = 18$)	NR ($n = 13$)
Proliferative index (%)*	45·2 (35·2–52·1)†	33·6 (19·2–49·1)	32·1 (22·1–48·1)
Survivin			
High	4	9	7
Low	5	9	6
bcl-2			
Positive	2	3	3
Negative	7	15	10
p53			
Positive	5	15	10
Negative	4	3	3

*Values are median (range). †$P = 0{\cdot}001$ (Kruskall–Wallis test)

Conclusion: The apoptotic genes studied were not molecular markers of response. However, these data provide evidence that highly proliferating tumours respond more favourably to cytotoxic agents.

Nuclear factor κB: relationship to Barrett's adenocarcinoma, stage and treatment response

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Background: Patients with Barrett's oesophagus are predisposed to oesophageal adenocarcinoma at higher rates than the general population. Nuclear factor (NF) κB has been shown to play a dominant role in cell physiology and control of apoptosis. The aim of this study was to examine the expression of NF-κB in oesophageal adenocarcinoma and its relationship to the effect of neoadjuvant chemoradiotherapy (CRT).

Methods: Mobility shift assay and Western blotting were used to measure NF-κB activity in nuclear extracts of fresh-frozen biopsies from tumour and uninvolved tissues (30 patients). Immunohistochemical staining of the NF-κB subunit RelA was also used to assess NF-κB activity (97patients).

Results: Tumour tissues expressed higher NF-κB levels than non-tumour tissues. CRT reduced NF-κB expression in oesophageal cancer. Reduced pre-CRT NF-κB expression correlated strongly with response to therapy (major response in NF-κB-negative group 75 per cent *versus* 17 per cent in the NF-κB-positive group; $P<0\cdot001$). Increased NF-κB levels were observed in Barrett's tissue adjacent to cancer.

Conclusion: These data show that oesophageal adenocarcinoma and Barrett's oesophagus are associated with increased NF-κB levels. The absence of NF-κB expression correlates with the early stage of the disease and predicts response to neoadjuvant therapy.

Cytogenetic analysis of patients with Barrett's oesophagus by interphase fluorescence *in situ* hybridization of oesophageal cells obtained by brush cytology

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Background: Previous cytogenetic data employing comparative genomic hybridization (CGH) have revealed amplifications of chromosomes 4 and 8. To confirm these findings, a study was undertaken of chromosome losses and gains using the complementary methodology of fluorescence *in situ* hybridization (FISH).

Methods: FISH was employed to study chromosome losses and gains in interphase Barrett's epithelial cells obtained by brush cytology. The epithelial cells were analysed using probes for the *p16* gene (9p21), the *Rb* gene (13q14) and the *p53* gene (17p13). In addition, centromeric chromosome probes were employed to study gain or loss of chromosomes 4, 8, 9 and Y.

Results: To date, 25 patients with Barrett's oesophagus with a range of premalignant conditions have been analysed. The findings suggest that chromosome 4 amplification is the earliest and most widespread alteration accompanying Barrett's progression. Other changes detected include chromosome 8 amplification, loss of the Y chromosome, and *p16* and *p53* gene deletions.

Conclusion: This study confirms previous CGH data by implicating the amplification of chromosomes 4 and 8. The widespread amplification of chromosome 4 in Barrett's progression may be a consequence of induced aneuploidy, or it may represent a specific genetic event that drives tumour formation.

Risk adjustment in oesophagogastric surgery: comparison of ASA and POSSUM regression models

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Background: Predictive models of operative mortality are increasingly applied in the evaluation of surgical performance. This study evaluated new prognostic models for the oesophagogastric population based on the American Society of Anesthesiologists (ASA) and Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM) methodology.

Methods: In-hospital mortality for 1042 patients undergoing oesophageal and gastric surgery from 35 UK hospitals between 1994 and 2000 was studied. Stepwise logistic regression (LR) models with split-sample validation were developed. Five-attribute O-POSSUM and ASA LR models were derived and compared using the independent co-variates: age, operative severity, mode of operation, staging and either physiological status (PS) or ASA. Observed : expected mortality rates (O : E ratio) were compared.

Results: The operative mortality rate was 12·0 per cent.

	Regression model	
	ASA LR	O-POSSUM
Development set (*n*=640)		
HL	14·98	14·50
C index	0·71	0·71
Validation set (*n*=402)		
HL	8·31	14·22
C index	0·72	0·75
Total (*n*=1042)		
O : E ratio	0·99	1·00

HL, Hosmer–Lemeshow statistic (low values indicate better model calibration); C index, area under the receiver–operator characteristic curve (high values indicate better model discrimination)

Conclusion: O-POSSUM offered better discrimination and accuracy, but the models predicted outcome equally well. The simplified ASA model offers an attractive alternative for units that wish to use ASA scores rather than POSSUM variables.

Preoperative evaluation of colorectal liver metastases using positron emission tomography

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Background: Colorectal liver metastases (CLMs) occur in up to half of all patients with colorectal cancer (CRC), but selection of patients for operative treatment of CLM is inaccurate when using conventional imaging modalities. This study was designed to investigate the role of positron emission tomography (PET), a metabolic imaging technique, in the preoperative evaluation of CLM.

Methods: Patients with CLM were recruited prospectively and underwent spiral computed tomography (CT) and fluorodeoxyglucose (FDG) PET within a 4-week interval. PET and CT scans were read independently, and the findings were compared as well as being correlated with tissue biopsy and/or clinical and radiological course (local ethics committee approval and informed patient consent were obtained).

Results: When using FDG-PET to evaluate the extent of disease and direct treatment, all 15 patients with multiple CLMs or extrahepatic spread were identified correctly (sensitivity and specificity 100 per cent). Spiral CT (sensitivity 53 per cent, specificity 100 per cent) failed to detect multiple CLMs in three of eight patients and distant extrahepatic metastases in four of seven patients. Information from FDG-PET altered clinical management in eight cases: three patients avoided inappropriate laparotomy, four commenced chemotherapy that would otherwise not have been delivered, and one patient underwent more radical surgery.

Conclusion: This study confirmed that PET is more accurate than CT for detecting CLMs and evaluating the presence of additional metastatic lesions. PET alters clinical management in up to one-third of patients, resulting in appropriate selection of patients for operative treatment and early treatment of those with distant metastatic disease, with a reduction in the number of inappropriate laparotomies.

Dynamic contrast-enhanced magnetic resonance imaging in investigation of indeterminate microcalcification in the breast

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Background: Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is an effective diagnostic modality for symptomatic breast disease, but its role in evaluating clinically occult disease associated with mammographically indeterminate microcalcification remains unclear. The aim of the study was to evaluate the efficacy of DCE-MRI in the investigation of indeterminate microcalcification in the breast.

Methods: Women with areas of indeterminate microcalcification on X-ray mammography had DCE-MRI examination of the breast. The data were evaluated morphologically and by postprocessing. Both signal intensity parameters and two-compartment pharmacokinetic modelling to calculate the amplitude of contrast uptake and exchange rate in the whole region of interest and within the most enhancing nine-pixel square were evaluated.

Results: Forty-two women were recruited. Histological diagnosis ($n = 37$) revealed: invasive disease ($n = 2$), ductal carcinoma *in situ* (DCIS) ($n = 10$), invasive disease plus DCIS ($n = 12$) and benign disease ($n = 13$). The remaining patients ($n = 5$) had benign findings on fine-needle aspiration cytology followed by clinical and mammographic follow-up for at least 15 (range 15–43) months. The mean (range) values for malignant *versus* benign lesions in arbitrary units for the maximum intensity time ratio were 42·70 (1·66–312·65) *versus* 9·79 (1·55–19·66) ($P = 0·002$). For exchange rate the values were 5·47 (0·25–49·26) *versus* 1·18 (0·27–2·31) ($P = 0·003$) and for amplitude of contrast uptake they were 8·61 (0·20–58·24) *versus* 1·19 (0·00–4·70) ($P = 0·007$).

Conclusion: DCE-MRI is able to distinguish benign from malignant clinically occult lesions associated with indeterminate microcalcification and is therefore a potentially useful investigation for this group of patients.

Genetic susceptibility in Dupuytren's disease

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Background: Dupuytren's disease (DD) is a familial condition of unknown cause affecting caucasians. Transforming growth factor (TGF) β family members such as TGF-β_1 and TGF-β_2 are key fibrogenic cytokines that are implicated in the pathogenesis of DD. Zf9 is a transcription factor that increases TGF-β_1 expression in tissue.

Methods: Novel single nucleotide polymorphisms (SNPs) were identified in the TGF-β family members by single-strand conformational polymorphism (SSCP), denaturing high-performance liquid chromatography (DHPLC) and by sequence alignment of gene sequence databases. Using a gene scanner and a polymerase chain reaction–restriction fragment length polymorphism method, multiple SNPs of the TGF-β_1, TGF-β_2, TGF-β receptor (TGF-βR) I, TGF-βRII, TGF-βRIIII and Zf9 gene were genotyped.

Results: A case–control association study of 289 patients with DD *versus* 255 caucasian controls demonstrated a statistically significant difference in genotype frequencies between cases and controls for a Zf9 gene polymorphism ($P = 0·007$). Presence of the G allele *versus* the A allele was associated with an increased risk of developing DD ($P = 0·002$).

Conclusion: This positive association may help to unravel the significant role of TGF-β in fibrosis. It may also explain the hereditary predisposition in DD. Understanding the genetic basis of DD is important for developing diagnostic and therapeutic regimens for a more efficacious treatment in the future.

Standard magnetic resonance imaging is inaccurate in selecting patients with rectal cancer for adjuvant therapy

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Background: Accurate information about nodal involvement and tumour penetration through the bowel wall is essential for selecting patients with rectal cancer for neoadjuvant chemotherapy and radiotherapy. Although several studies employing thin-slice techniques have shown that rectal tumours can be staged reliably by magnetic resonance imaging (MRI), the staging efficacy of standard MRI protocols has not yet been proven in a non-controlled clinical setting.

Methods: All MRI scans performed for rectal cancer staging between 1995 and 1999 were reviewed and the estimated Dukes' and tumour node metastasis (TNM) staging was compared with pathology results. The strength of agreement between MRI and pathology staging was determined with the kappa test.

Results: A total of 118 staging MRI scans for rectal cancer were analysed. The Dukes', T and N stages on MRI correlated with histopathology findings in 61, 63 and 74 per cent of cases respectively. The results of the kappa test for the degree of agreement between MRI and pathology results were $\kappa = 0{\cdot}69$ for Dukes' stage, $\kappa = 0{\cdot}29$ for T stage and $\kappa = 0{\cdot}46$ for N stage. MRI predicted tumour penetration of the rectal wall with 80 per cent sensitivity and 58 per cent specificity. Pelvic lymphadenopathy was identified with a sensitivity of 66 per cent and a specificity of 80 per cent. Tumour penetration through the rectal wall on MRI had a positive predictive value of 81 per cent and a negative predictive value of 57 per cent. The positive and negative predictive values for nodal involvement on MRI were 66 and 80 per cent respectively. The time elapsed between the MRI examination and surgery, and the tumour dimensions and position, had no influence on the accuracy of MRI staging.

Conclusion: These data suggest that standard MRI does not reliably estimate tumour rectal wall penetration and locoregional lymph node involvement, and therefore do not support its use for making decisions about the appropriateness of neoadjuvant chemoradiotherapy in patients with rectal cancer.

Technical skills examination for higher general surgical trainees

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Background: Assessing and comparing the technical skills of surgical trainees is a difficult task to accomplish objectively. An objective structured clinical examination (OSCE) is presented which enables stratification of the surgical skills of junior specialist registrars.

Methods: Twenty-two surgeons participated in the study, divided into basic surgical trainees (BST group; $n = 5$), junior specialist registrars (JSPR group; $n = 8$), senior specialist registrars (SSPR group; $n = 4$) and consultants (CON group; $n = 5$). BST and SSPR–CON groups acted as junior and senior controls respectively. They undertook a five-station OSCE, using three synthetic (bowel anastomosis, vascular anastomosis, saphenofemoral dissection) and two virtual reality-based (flexible sigmoidoscopy and laparoscopy) surgical simulations. Objective structured assessment of technical skill analyses and inbuilt computer scoring were used to measure individual performances. The Kruskal–Wallis test was used to determine the mean rank for each parameter, and the sum of the mean ranks used to generate a total score. The Mann–Whitney *U* test was used to observe differences between individual subgroups, and Cronbachs α analyses for intertest relationships.

Results: There was a significant improvement in overall performance with increasing seniority ($P < 0{\cdot}01$, Kruskal–Wallis test). Significant differences were observed between BST and JSPR groups ($P < 0{\cdot}02$) and between JSPR and SSPR groups ($P < 0{\cdot}05$) (Mann–Whitney *U* test). No significant differences were seen in SSPR *versus* CON groups. Within the JSPR group, the total mean rank range was 21·0–49·5. Cronbachs α reliability between all five tests was significant (0·81).

Conclusion: This examination successfully discriminated surgical skill, both between different grades of experience and within the target study group. High interstation correlation was also demonstrated.

Window of opportunity for timing emergency abdominal surgery

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Background: The Confidential Enquiry into Perioperative Deaths (CEPOD) guidelines recommend that all out of hours emergency abdominal surgery be deferred until the next daytime list, unless the condition is life threatening. Resuscitation is undertaken during this period but the optimal timing of intervention is not clear.

Methods: The first 28 patients admitted to the study presented with an abdominal emergency (peritonitis or obstruction) which after resuscitation was followed by operative intervention. Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM), Acute Physiology And Chronic Health Evaluation (APACHE) II and III, and Simplified Applied Physiology Score (SAPS II) were assessed at admission, during resuscitation and immediately before

operation. The level and site of monitoring and therapy were undertaken independently of the study.

Results: All scoring systems showed an improvement of predicted mortality following resuscitation. In some patients scores deteriorated again before operation, more so in the six patients who died after surgery. For example the median (range) POSSUM score at admission was 42 (17–51) in those who died and 29 (15–46) in survivors. After resuscitation, these scores fell to 34 (17–49) and 24 (15–45) respectively. Scores before operation were 45 (39–62) and 30 (17–46) ($P < 0{\cdot}002$, Mann–Whitney U test).

Conclusion: Scoring systems help to predict mortality and can indicate the optimal time for surgical intervention for abdominal emergencies.

Increased intestinal permeability and exaggerated cytokine response following ischaemia–reperfusion injury of critical limb ischaemia

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Background: An increase in intestinal permeability has been suggested to play an important role in development of the systemic inflammatory response syndrome (SIRS) after aortic surgery. The aim of this study was to investigate intestinal permeability and the inflammatory response following isolated lower-limb ischaemia–reperfusion (IR) injury.

Methods: Three groups of patients were studied. Group 1 comprised 18 patients with critical limb ischaemia (CLI) undergoing infrainguinal bypass surgery. Group 2 consisted of 14 patients with intermittent claudication undergoing infrainguinal bypass surgery, and group 3 comprised 12 patients with CLI unsuitable for arterial reconstruction who were undergoing major amputation. Intestinal permeability was assessed with the lactulose–mannitol differential urine excretion test (LM) before operation and on days 1, 3 and 7 after surgery. Plasma concentrations of endotoxin, urinary concentrations of p55 soluble tumour necrosis factor receptors (p55TNF-R) and SIRS scores were measured.

Results: A significant increase in mean(s.e.m.) LM ratio was observed in group 1 only on day 3 compared with the ratio observed before operation (0·04(0·03) *versus* 0·01(0·05); $P < 0{\cdot}05$, Wilcoxon signed ranks test). Endotoxin was not detected significantly during or after operation in any of the groups. Urinary p55TNF-R concentrations were significantly higher at day 1 in group 1 (62·1(30) ng ml^{-1}) compared with values in group 2 (20·0(32) ng ml^{-1}) and group 3 (23·7(84) ng ml^{-1}). After operation, 39 per cent of patients developed SIRS in group 1, compared with only 14 and 17 per cent in groups 2 and 3 respectively ($P < 0{\cdot}05$, χ^2 test).

Conclusion: These results confirm an increase in intestinal permeability associated with exaggerated cytokine response following IR injury in CLI only. They also suggest that the magnitude of IR injury is related to the degree of initial ischaemia.

Endothelial dysfunction and microalbuminuria precedes platelet function abnormalities in patients with insulin-dependent diabetes

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Background: Healthy endothelium plays a pivotal role in regulating haemostasis via the inhibition of platelet activation and aggregation. It was hypothesized that platelet dysfunction correlated with endothelial dysfunction in young patients with type 1 diabetes.

Methods: This hypothesis was tested in two groups of patients with type 1 diabetes. Group 1 comprised patients with normoalbuminuria and reversible impairment of flow-mediated dilatation. Patients in group 2 had microalbuminuria, indicating renal endothelial impairment.

Results: The results are shown in the *Table*. There were no significant difference between the groups (analysis of variance)

		Insulin-dependent diabetes mellitus	
Platelet parameter	Controls ($n=9$)	Normoalbuminuria ($n=12$)	Microalbuminuria ($n=8$)
CD62	14(2)	17(2)	10(2)
CD63	6(2)	5(1)	9(3)
GP1b	35 896(1878)	33 219(1286)	27 292(869)
Closure time (s) (as per PFA)	160(15)	162(15)	143(11)
Microparticles	4(1)	6(2)	2(1)

Values are mean(s.e.m.). All platelet values were within the normal reference range

Conclusion: The results demonstrate that antiplatelet therapy is not required in the early stages of type 1 diabetes and that endothelial dysfunction precedes and is a potential mediator of diabetic platelet dysfunction.

Role of urokinase-type plasminogen activator from bone marrow cells in the resolution of venous thrombi

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Background: Previous work has shown that venous thrombi in urokinase-type plasminogen activator-deficient (uPA$^{-/-}$) mice fail to resolve and are associated with a reduced early monocyte infiltration. The aim of this study was to determine the importance of bone marrow-derived cells, particularly monocytes (a source of urokinase), in the resolution of venous thrombi using bone marrow transplantation in uPA$^{-/-}$ mice.

Methods: Two groups of uPA$^{-/-}$ mice and two groups of wild-type mice ($n = 14$ per group) were irradiated to deplete their bone marrow. Each group of uPA$^{-/-}$ and wild-type mice was transplanted with bone marrow from either wild-type mice or uPA$^{-/-}$ mice. Thrombi were induced in the infrarenal vena cava 4 weeks after transplantation and then harvested after a further 4 weeks. Serial transverse paraffin-fixed sections were taken at 250-µm intervals along the entire length of each thrombus and stained with Martius Scarlet Blue. Thrombus size (total cross-sectional area) was measured using computer-assisted image analysis. (All animal experiments were carried out in Leuven, Belgium.)

Results: uPA$^{-/-}$ mice transplanted with wild-type marrow resolved their thrombi to a mean(s.e.m.) size of 0·3(0·1) mm^2, whereas thrombi in the uPA$^{-/-}$ mice transplanted with uPA$^{-/-}$ marrow were significantly larger (3·9(1·1) mm^2) ($P < 0{\cdot}001$, Mann–Whitney U test). A similar trend was observed in wild-type mice transplanted with marrow from either wild-type or uPA$^{-/-}$ mice (0·8(0·2) *versus* 2·0(0·6) mm^2 respectively; $P = 0{\cdot}2$).

Conclusion: Transplantation of uPA$^{-/-}$ mice with bone marrow from wild-type mice promoted venous thrombus resolution, suggesting a key role for uPA from bone marrow-derived cells.

Adenosine 5′-diphosphate : adenosine 5′-triphosphate ratios in patients undergoing abdominal aortic aneurysm surgery: a rapid measure of cellular stress

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Background: During cellular stress, oxidative phosphorylation may become compromised resulting in altered adenosine 5′-diphosphate (ADP) and adenosine 5′-triphosphate (ATP) metabolism. Here, ADP : ATP ratios were measured as a rapid marker of cellular stress *in vivo* during abdominal aortic aneurysm (AAA) repair.

Methods: Twenty-two consecutive patients undergoing elective AAA surgery were studied prospectively: 12 had open repair (OR) and ten had endovascular repair (ER). Peripheral vein (PV) and femoral vein (FV) blood samples were obtained before operation, at the end of ischaemia and at 5 min, 4, 24, 48, 72 and 144 h after reperfusion (PV only at baseline, 72 and 144 h). Serum ADP : ATP ratios were measured by means of a bioluminescence technique (ApoGlow$^{\text{TM}}$, Lumitech$^{\text{TM}}$, Cambrex, East Rutherford, New Jersey, USA).

Results: In patients who had OR the median ratio increased significantly from the preoperative level to that measured at end-ischaemia in both PV (2·5 *versus* 8·3, $P = 0{\cdot}003$) and FV (2·5 *versus* 5·0, $P = 0{\cdot}05$). In patients who had ER there was a smaller and non-significant rise in median ratio values at end-ischaemia. In both OR and ER groups the median ratio values returned to preoperative levels over 24–72 h of reperfusion. At 144 h after reperfusion there was a significant secondary rise in median ratio values from 24 and 48 h after reperfusion in ER and OR patients respectively (ER: 0·78 *versus* 3·63, $P = 0{\cdot}008$; OR: 0·76 *versus* 5·3, $P = 0{\cdot}05$).

Conclusion: Raised ADP : ATP ratios were seen during maximal tissue ischaemia and surgical injury with a second peak at 144 h. An increasing body of evidence suggests that increasing ratios are a marker of apoptosis, and the second peak illustrated in this study may reflect this pathophysiological process.

Improving endothelial cell retention in seeding with arginine–glycine–aspartate- and heparin-bonded grafts under physiological shear stress

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Background: Endothelial seeding has been shown to improve the patency of prosthetic grafts. This study investigated whether this could be further improved by bonding of the graft lumen with arginine–glycine–aspartate (RGD), heparin or both on to a compliant polyurethane (MyoLink$^{\text{TM}}$, Credent Vascular Technologies, Wrexham, UK) graft.

Methods: Human umbilical vein endothelial cells were cultured, radiolabelled with indium-111 oxine, seeded on to RGD-, heparin- or RGD–heparin-bonded MyoLink$^{\text{TM}}$ grafts, and exposed to physiological flow and shear stress for 6 h. Cell retention was assessed with a γ camera and the experiment was repeated six times.

Results: The *Table* shows percentage cell retention at 1, 4 and 6 h.

Graft	1 h	4 h	6 h
PTFE	56(6)	44(3)	42(8)
MyoLink$^{\text{TM}}$	69(11)	65(9)	61(10)*
Heparin + MyoLink$^{\text{TM}}$	78(9)	70(6)	68(9)*
RGD + MyoLink$^{\text{TM}}$	80(1)	73(4)	71(2)*
RGD + heparin + MyoLink$^{\text{TM}}$	85(1)	78(2)	76(2)*†

Values are mean(s.d.). PTFE, polytetrafluoroethylene. *$P < 0{\cdot}05$ *versus* PTFE; †$P < 0{\cdot}05$ *versus* MyoLink$^{\text{TM}}$ (Kruskal-Wallis test)

Conclusion: RGD–heparin-bonded Myolink$^{\text{TM}}$ is significantly better than PTFE and native MyoLink$^{\text{TM}}$ in improving cell retention, and provides an antithrombogenic surface. The potential of single-stage seeding using this RGD–heparin-bonded graft shows clinical promise.

Excess molecular oxygen reduces the production of reactive oxygen species in minor ischaemia–reperfusion

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Background: The availability of molecular oxygen upon reperfusion results in the acute generation of reactive oxygen species (ROS) responsible for ischaemia–reperfusion injury (IRI). It is not known whether giving oxygen supplements to patients with IRI increases the reperfusion damage. The aim of this study was to investigate the effect of oxygen on the total antioxidant capacity and red cell glutathione levels during an ischaemia–reperfusion episode induced by claudication.

Methods: Six normal subjects and six patients (claudicants) with symptomatic peripheral arterial disease were exercised to fatigue (normal subjects) or to the limit of maximal pain (claudicants). Venous blood samples were taken before and 1 min after exercise. Claudicants repeated the same test while breathing 100 per cent oxygen at 2·4 atmospheres of pressure.

Results: Mean(s.e.m.) red cell glutathione levels were significantly reduced after exercise in claudicants, from 1211(425) to 1069(435) μmol l^{-1} ($P = 0{\cdot}043$, Wilcoxon test). The levels in normal subjects increased from 1526(229) to 1727(55) μmol l^{-1} ($P = 0{\cdot}032$, Mann–Whitney U test). After exercise while breathing oxygen, a paradoxical increase in mean(s.e.m.) red cell glutathione levels (compared with breathing air) was observed in claudicants, rising from 1350(552) to 1502(307) μmol l^{-1}. A similar trend was observed in the total antioxidant capacity, although this was not statistically significant.

Conclusion: Oxygen supplementation during ischaemia–reperfusion induced by claudication resulted in a significant reduction in the generation of reactive oxygen species upon reperfusion. This should reduce the reperfusion injury and may therefore have important therapeutic implications.

Enhancement of infrainguinal arterial bypass graft flow with intermittent pneumatic compression

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Background: As intermittent pneumatic compression (IPC) increases arterial leg inflow, it was hypothesized that it might enhance infrainguinal arterial graft flow. This study examined the immediate effects of IPC applied to foot (IPC$_{\text{foot}}$), calf (IPC$_{\text{calf}}$) and both simultaneously (IPC$_{\text{foot+calf}}$) on infrainguinal graft haemodynamics.

Methods: Eighteen femoropopliteal and 18 femorodistal grafts, with resting ankle : brachial pressure indices of 0·9 or more and no graft stenosis greater than 50 per cent, had mean (mV), peak systolic (PSV) and end diastolic (EDV) velocities, pulsatility index (PI) and flow (Q) measured at mid-to-distal thigh on sitting, at rest and within 5 s of IPC using duplex ultrasonography. IPC sequence was randomly assigned in a cross-over design (120 mmHg per 4 s; three cycles per min).

Results: Data are presented as the percentage change from baseline.

	Q	mV	PSV	PI
IPC$_{\text{foot+calf}}$				
Femoropopliteal	293	262	55	−56
	(181–378)	(162–332)	(21–90)	(−44–67)
Femorodistal	218	202	55	−55
	(128–346)	(106–315)	(22–74)	(−47–67)
IPC$_{\text{calf}}$				
Femoropopliteal	243	231	32	−36
	(145–295)	(137–271)	(23–65)	(−30–68)
Femorodistal	192	170	34	−40
	(83–411)	(77–284)	(20–52)	(−30–57)

Values are median (interquartile range). All $P < 0{\cdot}005$

With IPC$_{\text{foot}}$ Q, mV and PSV increased significantly in both grafts ($P < 0{\cdot}01$). EDV with IPC increased significantly ($P < 0{\cdot}005$). Haemodynamics in femoropopliteal and femorodistal grafts did not differ ($P > 0{\cdot}1$).

Conclusion: IPC$_{\text{foot}}$, IPC$_{\text{calf}}$ and IPC$_{\text{foot+calf}}$ proved particularly effective in augmenting infrainguinal graft flow, the associated increase in EDV and the decrease in PI, implicating peripheral resistance attenuation. By assisting graft flow, IPC may help to reduce the risk of graft thrombosis.

Effect of exercise training on flow-mediated dilatation in patients with intermittent claudication

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Background: Exercise training may improve endothelial dysfunction in patients with atherosclerosis. Flow-mediated dilatation (FMD) of the brachial artery is a novel method of measuring endothelial function; this study aimed to determine the effect of exercise training on FMD in patients with intermittent claudication.

Methods: Patients with stable symptoms of intermittent claudication were randomized to either 3 months of supervised exercise or a control group receiving exercise advice alone. All patients continued their current medications without dose modification. Flow-mediated dilatation was assessed before randomization, and after 3 months and 6 months of exercise training using a well characterized non-invasive technique by means of high-resolution ultrasonography (10 MHz). Endothelium-dependent flow-mediated responses were assessed during a hyperaemic response to arterial occlusion, and endothelium-independent dilatation was assessed in response to 400 µg sublingual glyceryl trinitrate (GTN) spray.

Results: Sixty patients were randomized, with 53 successfully followed at 3 months. Baseline responses were similar in both groups ($P = 0·21$, Mann–Whitney U test). Following exercise training there were no significant changes in flow-mediated dilatation or dilatation to GTN in either group.

	Baseline	3 months
Flow-mediated dilatation		
Supervised	3·8 (1·3, 5·0)	3·5 (2·5, 6·1)
Control	1·9 (−0·2, 4·8)	1·8 (0·3, 4·7)
GTN dilatation		
Supervised	13·8 (11·1, 21·8)	21·8 (12·7, 28·1)
Control	13·7 (10·8, 25·8)	17·3 (11·8, 22·4)

Values are percentages with 95 per cent confidence intervals in parentheses

Conclusion: Exercise training for intermittent claudication did not improve flow-mediated dilatation.

Pulmonary injury due to ischaemia–reperfusion is ameliorated by hypertonic saline infusion

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Background: The inhibition of neutrophil–endothelial cell interactions and macrophage function by hypertonic saline (HTS) may confer protection against organ injury in states of immunological disarray. This study tested the hypothesis that infusion of HTS modulates the development of end-organ injury in a model of lower-torso ischaemia–reperfusion injury (IRI).

Methods: IRI was induced in 24 male Sprague–Dawley rats by infrarenal aortic cross-clamping for 30 min followed by reperfusion for 2 h. At 0 and 60 min reperfusion, intravenous HTS (7·5 per cent sodium chloride 4 ml kg^{-1}) was administered to six rats, while a further six animals received normal saline 4 ml kg^{-1}. Six rats received HTS 4 ml kg^{-1} immediately before the induction of ischaemia. At 72 h, pulmonary pathology, neutrophil infiltration (myeloperoxidase (MPO) activity) and endothelial permeability (bronchoalveolar lavage (BAL) and wet : dry weight ratios) were assessed.

Results: HTS infusion before or after ischaemia reduced pulmonary oedema significantly (*Table*). In addition, a significant reduction in pulmonary injury scores based on histological criteria was observed ($P < 0·05$).

	Lung MPO activity (units g^{-1})	BAL protein content	Lung wet : dry weight ratio
HTS	1·54(0·19)*	0·02(0·00)*	2·06(0·26)*
HTS pretreated	1·18(0·12)*	0·02(0·00)*	1·25(0·07)*
Normal saline	2·07(0·23)	0·06(0·01)	4·68(0·44)
Control	1·17(0·02)	0·01(0·00)	1·09(0·20)

Values are mean(s.e.m.). *$P < 0·05$ *versus* normal saline group (Kruskal–Wallis one-way analysis of variance on ranks)

Conclusion: Resuscitation with HTS attenuates the pulmonary oedema and tissue injury resulting from lower-torso ischaemia–reperfusion.

Leucocyte depletion and postoperative infection: a multicentre randomized controlled trial

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Background: Postoperative infection causes unnecessary morbidity. While the causes are multifactorial, there is evidence that the immunosuppressive effects of allogeneic blood transfusion may predispose to infection.

Methods: A prospective randomized multicentre study of transfusion with buffy coat-poor (BCP) or leucocyte-depleted (LD) blood was undertaken in patients undergoing major surgery. The Welsh blood transfusion service prepared all blood products. Postoperative infection was classified using internationally recognized criteria and mortality up to 12 weeks following the initial operation was counted. Total costs for each group were also calculated.

Results: Patients were recruited between May 1998 and April 2000; a total of 559 were available for analysis. Some 279 patients were randomized to receive BCP blood and 280 to receive LD blood. The non-transfused (control) group comprised 119 patients (43 per cent) randomized to BCP and 137 (49 per cent) to LD. Not surprisingly, transfused patients had an immediate postoperative infection rate significantly higher than that in non-transfused patients (43 *versus* 18 per cent). The variable (type of transfusion) showed a non-significant lower trend in the rate of infection in LD patients (50 per cent for BCP *versus* 38 per cent for LD). Reoperated patients experienced a higher incidence of infection in the BCP group (15 per cent for BCP *versus* 7 per cent for LD; $P = 0·04$). The benefits of LD blood became apparent in those receiving 2 units or more (infection rate of 60 per cent for BCP *versus* 39 per cent for LD; $P = 0·01$) and in operations taking less than 200 min. There was a lower incidence of infection in the LD group (47 per cent for BCP *versus* 27 per cent for LD). In patients undergoing gastrointestinal surgery, the mortality rate was significantly greater in the BCP than in the LD group (20 *versus* 6 per cent respectively; $P = 0·03$). Earlier discharge following infection in LD patients reduced the cost of hospital occupancy by an average of £640 per patient.

Conclusion: Leucodepletion reduces infection and mortality in patients undergoing major surgery, but benefits are reduced when the operation is prolonged. Significant cost differences occurred following infection and warrant the use of LD blood products.

Hepatic B cells: a role in rejection following human orthotopic liver transplantation?

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Background: Normal human liver contains a unique subset of lymphocytes that may influence hepatic immune responses. These lymphocytes can be transferred from donor to recipient during orthotopic liver transplantation (OLT). The aim of this study was to investigate whether the numbers of these T and B 'passenger' lymphocytes in the donor liver are associated with the occurrence and severity of acute cellular rejection (ACR) and to study their variation during ACR following human OLT.

Methods: Donor liver tissue taken from 17 adult donors before transplantation was stained by immunohistochemical methods for CD3 (T cells) and CD20 (B cells). Positively stained cells in the portal tract and parenchyma were identified by light microscopy. Clinically diagnosed episodes of rejection were confirmed by histological examination of post-transplantation biopsies. Eighteen biopsies from 12 patients who had ACR following OLT were also stained by immunohistochemical means for T and B cells.

Results: Five patients had no evidence of ACR, six had one rejection episode, four had two episodes and the other two patients had three and four episodes. There was a significant correlation between the number of B lymphocytes in the parenchyma of the donor livers and the number of episodes of rejection ($P = 0{\cdot}02$) and the severity of rejection ($P = 0{\cdot}04$). As expected there was an increase in the numbers of CD3-positive T cells in corresponding rejection biopsies; however, there was a significant decrease in the numbers of CD20-positive B cells in the parenchyma of these biopsies ($P = 0{\cdot}003$).

Conclusion: At the time of OLT, higher numbers of B lymphocytes are present in donor liver destined subsequently to develop ACR. However, B lymphocyte numbers are reduced in the presence of ACR. Further studies are needed to explain the relationship between B lymphocytes and ACR.

Evidence for specific cytotoxic T lymphocyte immunity against telomerase in patients with colorectal cancer

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Background: Characterization of the cytotoxic T lymphocyte (CTL) activity against tumour antigens is crucial for understanding cancer immune surveillance mechanisms. Although telomerase activity is found in 90–100 per cent of colorectal cancers, there are few data on the prevalence of specific antitelomerase immunity.

Methods: The CTL response against two human leucocyte antigen (HLA) A2-restricted epitopes of human telomerase reverse transcriptase (hTERT) was studied *ex vivo* in 14 patients with HLA-A2+ colorectal cancer and four healthy subjects. An interferon (IFN) γ Elispot assay (R & D Systems Europe, Abingdon, UK) was used to quantify the amplitude of the specific CTL response against telomerase, following incubation of peripheral blood mononuclear cells (PBMCs) with hTERT peptides. PBMCs incubated with or without phorbol 12-myristate-13-acetate (PMA) served as positive and negative controls respectively. The levels of the specific CTL response against HLA-A2-restricted peptides of influenza A and carcinoembryonic antigen (CEA) were also determined to allow comparison.

Results: Specific CTLs against one of the two hTERT peptides were found in six of the 14 patients with colorectal cancer, with four of these recognizing both peptides. The frequencies of telomerase-specific CTLs were between 15 and 45 per 10^6 PBMCs. In addition, ten patients had CTLs that were reactive with the influenza A peptide, and five had CTLs that responded to stimulation with the CEA peptide. CTLs active against telomerase were found both in patients with disseminated disease, as well as in patients with early disease. None of the healthy volunteers studied presented CTLs that were capable of recognizing telomerase and/or CEA antigens.

Conclusion: This study demonstrates that patients with colorectal cancer are capable of mounting a specific cellular immune response against telomerase antigens. These findings suggest that the lack of an effective immune response against tumour cells that possess telomerase activity is not due to the absence of CTLs directed against telomerase epitopes, but probably to a defective presentation mechanism for these antigens.

Peritoneal adhesion formation attenuated by thermal preconditioning while maintaining laparotomy wound and gut anastomotic integrity

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Background: Thermal preconditioning (TPC) modulates cellular responses to injury. However, the effect on repair is unknown. Postoperative adhesion formation is the commonest cause of small bowel obstruction in the Western world.

Methods: The aims of this study were to assess the role of TPC in preventing intra-abdominal adhesions and its possible adverse effect on wound healing. Sprague–Dawley rats ($n = 34$) were randomized into two groups: a TPC group, in which animals received TPC 18 h before surgery and a control group. Laparotomy wound tensile strength and colonic anastomosis bursting pressure (CABP) were assessed as measures of wound healing. The degree of adhesions was assessed according to a recognized adhesion scoring system.

Results:

	Control	TPC	P^*
Adhesion score	4·38(0·7)	2·62(1·2)	0·01
Tensile strength	1177(119)	1037(195)	0·54
CABP	82·3(14)	108·6(30)	0·40

Values are mean(s.e.m.). *Analysis of variance

Conclusion: These results provide proof for the concept that TPC attenuates peritoneal adhesion formation with no adverse effect on healing. Evaluation of pharmacological preconditioning strategies is now warranted prior to clinical application.

Endogenous adenosine attenuates the cytokine response to endotoxin

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Background: Endotoxin induces a systemic inflammatory response, leading to inflammatory-mediated tissue injury and organ dysfunction. It was hypothesized that R75231, by inhibiting the metabolism of endogenous adenosine, would ameliorate endotoxin-induced multiorgan dysfunction by attenuating such a systemic inflammatory response.

Methods: With Home Office approval, anaesthetized swine underwent a laparotomy and insertion of a sigmoid tonometer. The control group ($n = 7$) received no further insult. Remaining animals ($n = 12$) received *Escherichia coli* endotoxin (lipopolysaccharide (LPS) 50 μg kg^{-1}) with or without R75231 pretreatment (0·3 mg kg^{-1} via a gastrostomy tube at laparotomy). Blood was taken for blood gas analysis and determination of chemiluminescent phagocyte respiratory burst priming. Plasma concentrations of tumour necrosis factor (TNF) α and interleukin (IL) 8 and IL-10 were assayed by enzyme-linked immunosorbent assay. The alveolar–arterial (A-a) oxygen gradient (increased with impaired oxygen transport) and sigmoid pH were recorded as markers of organ injury.

Results:

	T	Control	LPS	LPS + R75231
TNF-α (ng ml^{-1})	75	0·0 (0·0–0·3)	8·0 (7·0–10·0)*	4·5 (3·1–7·3)*
IL-8 (pg ml^{-1})	75	36 (21–197)	98 (89–125)	33 (24–55)†
IL-10 (ng ml^{-1})	75	0·0 (0·0–0·3)	1·5 (1·3–1·6)*	1·2 (0·9–1·3)*
Priming (%)	15	23 (17–41)	51 (42–73)*	65 (31–73)*
A-a (mmHg)	270	494 (283–544)	573 (558–589)*	566 (551–593)*
Sigmoid pH	180	7·34 (7·31–7·56)	7·23 (7·19–7·26)*	7·25 (7·20–7·33)

Values are median (interquartile range). T, time in mins. *$P < 0\cdot05$ *versus* control; †$P < 0\cdot05$ *versus* LPS (Mann–Whitney *U* test)

Conclusion: Endotoxin-induced pulmonary and intestinal injury is associated with a cytokine cascade and phagocyte priming. R75231 pretreatment attenuates the cytokine response but does not ameliorate organ dysfunction.

Tumour necrosis factor α enhances tumour cell adhesion to the mesothelium via upregulation of intercellular adhesion molecule 1: a mechanism by which surgical trauma increases tumour recurrence

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Background: Approximately 25–35 per cent of patients who develop recurrent colorectal cancer present with localized peritoneal metastases, and it is known that increased surgical trauma enhances tumour metastasis and peritoneal recurrence. The hypothesis was that the inflammatory cytokine tumour necrosis factor (TNF) α, which is a marker of surgical trauma, enhances tumour–mesothelial interactions via an upregulation of intercellular adhesion molecule (ICAM) 1.

Methods: Monolayers of primary cultures of human peritoneal mesothelial cells (HPMCs) cultured in 96-well plates were incubated in TNF-α 5 ng ml^{-1} for 4 h. Adherence of a colonic adenocarcinoma cell line, SW1222, to the HPMCs was assessed using a fluorometric adhesion assay and compared with a control. Mesothelial cells were then incubated in TNF-α 5 ng ml^{-1} for 4 h or in standard conditions and cell surface receptor expression, in particular that of ICAM-1, was assessed in both groups using flow cytometry. The adhesion assay was then repeated using an inhibitory monoclonal (Mab) antibody to ICAM-1.

Results: TNF-α enhanced tumour cell adhesion to HPMCs (mean(s.e.m.) percentage adhesion 45·76(2·40) for TNF-α *versus* 22·80(1·61) for control; $P < 0\cdot001$, analysis of variance). This was associated with an upregulation of ICAM-1 expression (mean(s.e.m.) fluorescence 996·17(100·7) *versus* 671·51(69·0) for control; $P = 0\cdot03$, analysis of variance). The enhanced adhesion observed following incubation with TNF-α was attenuated by an inhibitory ICAM-1 Mab: $P = 0\cdot005$ (TNF-α alone *versus* TNF-α and ICAM-1 Mab, two-way analysis of variance).

Conclusion: TNF-α enhances tumour cell adhesion to a mesothelial cell monolayer via an upregulation of ICAM-1, demonstrating a mechanism by which surgical trauma enhances tumour metastasis.

Nitric oxide pathway in critical limb ischaemia

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Background: Nitric oxide plays an important role in the regulation of local vasomotor tone, with evidence that systemic nitric oxide formation rates are decreased in patients with peripheral vascular disease. This study investigated changes in the nitric oxide pathway in patients with critical limb ischaemia (CLI).

Methods: Following local ethics committee approval, blood samples and lower-limb muscle biopsies were taken from six patients with CLI and from six surgical patients with no lower-limb ischaemia (controls). Plasma nitrate and nitrite levels were measured by chemiluminescence to reflect systemic plasma nitric oxide levels. Nitric oxide synthase (NOS) distribution on muscle sections was studied using reduced nicotinamide adenine dinucleotide phosphate-diaphorase (NADPHd) histochemistry and immunohistochemistry with antiendothelial NOS (eNOS) antibodies. eNOS messenger RNA (mRNA) levels in muscle homogenates were measured using real-time quantitative reverse transcription-polymerase chain reaction, and NOS activity of muscle biopsies was assessed with the citrulline assay.

Results: Plasma nitrate and nitrite levels were increased in patients with CLI compared with controls (mean(s.e.m.) 9·50(1·67) µm *versus* 3·58(1·05); $P < 0·05$, Mann–Whitney *U* test). NADPHd staining was increased in CLI sections and was associated with muscle fibres and microvessels. eNOS mRNA expression was increased in CLI muscle ($P < 0·05$) and the eNOS isoenzyme was associated mainly with microvessels. However, there was no evidence of increased NOS activity in CLI muscle compared with control.

Conclusion: These results suggest that, while plasma nitric oxide levels and tissue NOS mRNA and protein levels were raised in CLI muscle, NOS activity and therefore nitric oxide availability in the ischaemic muscle was not increased. This could represent an inadequate attempt to improve perfusion to ischaemic muscle via the nitric oxide pathway.

Effect of hyperoxia on regional blood flow in diabetic patients with peripheral arterial disease

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Background: Arteries are sensitive to changes in oxygen tension. However, various vascular beds react differently to oxygen. This study investigated the systemic effects of oxygen on regional flow in the extremities of diabetic and non-diabetic patients with arterial disease.

Methods: Sixteen diabetic and five non-diabetic patients with peripheral arterial disease, and six normal controls were recruited. Cutaneous laser Doppler flux and transcutaneous oximetry were carried out in the forearm at rest and after breathing 100 per cent oxygen. Regional flow was measured by heating the laser Doppler probe to 44°C.

Results: After breathing 100 per cent oxygen, mean(s.e.m.) transcutaneous oxygen tension increased from 53(10) to 137(54) mmHg in the diabetic patients, from 55(9) to 168(62) mmHg in non-diabetic patients, and from 65(18) to 246(88) mmHg in controls. In contrast, cutaneous blood flow was reduced by 16(14), 12(22) and 5(30) per cent respectively. However, regional blood flow increased by 10(36) per cent in the control group and by 14(32) per cent in non-diabetic patients. In comparison, regional blood flow in the diabetic group increased by 96(31) per cent ($P = 0·020$, Kruskal–Wallis test).

Conclusion: Diabetic patients with peripheral arterial disease respond distinctly to inspired oxygen with a profound increase in regional blood flow to the extremities. This effect of oxygen may have important therapeutic implications in critical ischaemia and ischaemic ulceration.

Urinary nitrate excretion correlates with urinary interleukin 1β after vascular surgery

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Background: *In vitro* models have shown that renal proximal tubular injury is related to cytotoxic levels of nitric oxide produced by inducible nitric oxide synthase (iNOS) induction and mediated by proinflammatory cytokines. The urinary proinflammatory cytokine interleukin (IL) 1β may therefore correlate with renal nitric oxide production in an *in vivo* model of hind-limb ischaemia–reperfusion.

Methods: Twelve pigs underwent laparotomy, normodynamic fluid resuscitation for 2 h, bilateral hind-limb ischaemia for 2 and 2·5 h of reperfusion. Urine samples were collected after 30 min and 2, 3, 4, 5 and 6·5 h following induction of anaesthesia. Urinary nitrate and IL-1β levels were measured by enzyme-linked immunosorbent assay. Correlational analysis was with the Spearman rank test after logarithmic transformation.

Results: There was a correlation between urinary IL-1β concentration in the 30-min sample and urinary nitrate levels in the 2-h sample ($r^2 = 0·56$, $P < 0·002$). Histological evidence of acute tubular necrosis was evident in all animals, although plasma urea and creatinine levels remained normal throughout.

Conclusion: Urinary IL-1β may influence intrarenal nitrate production, possibly through proximal tubular iNOS activity. This *in vivo* porcine model has potential in elucidating the possible role of nitric oxide in early renal injury after major vascular surgery.

Appropriate use of carotid duplex imaging: results of a prospective audit

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Background: In recent years there has been an increased demand for carotid duplex imaging, but it is not known whether this has resulted in an increased detection of significant (70 per cent) internal carotid artery (ICA) lesions.

Methods: A prospective audit of carotid duplex scans in a university-affiliated teaching hospital was carried out using data entered in the computerized vascular database. Each scan request was analysed for source of referral, indication and scan result. Some 2151 new patients were scanned in a 25-month period beginning in June 1999. A positive scan was any scan that detected more than 70 per cent stenosis in either ICA.

Results: The overall detection of significant ICA lesions was 16·5 per cent. Vascular surgeons requested 343 scans and had the highest positive scan rate of 25·4 per cent. General physicians, geriatricians, cardiologists and ophthalmologists had rates ranging from 15·0 to 18·7 per cent. Neurologists were worst at detecting significant ICA lesions (5·9 per cent). This was attributed to a newly created nurse-led transient ischaemic attack clinic, where the interpretation of patient symptoms was often incorrect. Asymptomatic bruit (32·6 per cent), amaurosis fugax (18·9 per cent) and stroke (18·5 per cent) were the indications associated with the highest proportion of positive scans.

Conclusion: For a carotid duplex service to be financially viable, it should have a reasonably successful pick-up rate of ICA disease. The correct interpretation of vague and uncommon symptoms is crucial to achieve this and to avoid a large number of unnecessary scans.

Nitric oxide mediates the attenuation of homocysteine–adenosine-induced apoptosis by vitamin B_{12} supplementation

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Background: Homocysteine (Hcy) in combination with adenosine induces apoptosis of human umbilical vein endothelial cells (HUVECs), which can be prevented by vitamins B_{12}, B_6 and folate. The aim of this study was to determine whether nitric oxide mediates the antiapoptotic effect of vitamin B_{12} supplementation.

Methods: HUVECs and long saphenous vein endothelial cells (LSVECs) were co-incubated with D,L-Hcy, adenosine, methylcobalamin (B_{12}) and *N*-monomethyl-L-arginine (L-NMA), a specific nitric oxide synthase (NOS) inhibitor, either alone or in combination for 4 h. Apoptosis and nitric oxide levels were measured by flow cytometry after staining with annexin V, propidium iodide and a nitric oxide fluorescent probe.

Results: Hcy, adenosine, B_{12} and L-NMA alone did not induce significant apoptosis compared with the control. Vitamin B_{12} increased nitric oxide levels significantly and this was associated with a marked attenuation of apoptosis. Inhibition of NOS with L-NMA did not attenuate the rise in nitric oxide caused by B_{12}, indicating that the latter acts as an exogenous nitric oxide donor.

	Nitric oxide*	Apoptosis (%)
Control	100	8(2·2)
Hcy + adenosine	85(3·2)†	22(5·2)†
Hcy + adenosine + B_{12}	182(9·8)‡	9(3·5)‡
Hcy + adenosine + B_{12} + L-NMA	178(6·1)‡	10(2·6)‡

Values are mean(s.d.); *percentage of control value. Adenosine concentration 0·5 mmol l^{-1}; B_{12} concentration 0·1 mmol l^{-1}; L-NMA concentration 0·5 mmol l^{-1}. †$P < 0·01$ *versus* control, ‡$P < 0·01$ *versus* Hcy + adenosine (analysis of variance)

Conclusion: Vitamin supplementation increases nitric oxide concentration, independently of NOS, in HUVECs and LSVECs incubated in Hcy plus adenosine. The rise in nitric oxide levels seen with vitamin B_{12} was associated with a significant antiapoptotic effect and may be an important mechanism by which vitamin B_{12} protects endothelium from the adverse effects of homocysteine.

Effect of exercise training for intermittent claudication on cardiac risk factors and inflammatory mediators

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Background: Patients with intermittent claudication suffer enhanced cardiovascular and cerebrovascular morbidity and mortality. The aim of this study was to investigate the effect of exercise training on cardiac risk factors and inflammatory markers in patients with intermittent claudication.

Methods: Patients with stable symptoms of intermittent claudication were randomized to either 3 months supervised exercise or a control group receiving exercise advice alone. All patients continued their current medications without dose modification. Patients were assessed before randomization and at 3 and 6 months when fasting blood samples were taken for measurement of lipid profile, levels of homocysteine, glucose, haemoglobin A1c and C-reactive protein (CRP), and plasma viscosity (PV). Neutrophil CD11b expression before and after treadmill exercise was measured by flow cytometry of whole blood. Statistical analysis was with the Mann–Whitney *U* test.

Results: Sixty patients were randomized, with 53 patients successfully followed at 3 months. Supervised exercise increased high density lipoprotein (HDL) cholesterol (median 0·0 (interquartile range 0·0–9·9) per cent) and reduced low density lipoprotein cholesterol in the supervised group compared with controls (median –6·1 *versus* 4·7 per cent; $P = 0{\cdot}04$). This produced a significant reduction in total/HDL cholesterol (–5·3 *versus* 1·9 per cent; $P = 0{\cdot}02$). No significant changes in the levels of fasting triglycerides, total cholesterol, blood glucose, HbA1c, homocysteine or the inflammatory markers CRP and plasma viscosity were detected following exercise training. Acute exercise increased neutrophil CD11b expression, but these levels were not affected by exercise training.

Conclusion: Supervised exercise training for intermittent claudication favourably modified the fasting lipid profile and may thus be of benefit in the secondary prevention of cardiovascular and cerebrovascular events.

Is nitric oxide metabolism deranged following conventional abdominal aortic aneurysm surgery?

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Background: Peroxynitrite is the product of a diffusion-limited reaction between superoxide and nitric oxide, and has been proposed as a mediator of tissue injury during reperfusion of ischaemic tissues. Nitrotyrosine (NT) was measured as a stable marker of peroxynitrite production *in vivo* during abdominal aortic aneurysm (AAA) repair.

Methods: Twenty-two consecutive patients undergoing elective AAA surgery were studied prospectively: 13 had open repair and nine had endovascular repair. Peripheral vein (PV) and femoral vein (FV) blood samples were obtained before operation, at the end of ischaemia, and at 5 min, 4, 24, 48, 72 and 144 h following reperfusion (PV only at baseline, 72 and 144 h). Plasma NT concentrations were measured with an enzyme-linked immunosorbent assay.

Results: In patients who had open repair, median NT concentrations increased significantly from preoperative levels to 5 min of reperfusion in both PV (0·43 *versus* 0·59 nmol per mg protein; $P = 0{\cdot}004$) and FV (0·43 *versus* 0·61 nmol per mg protein; $P = 0{\cdot}002$). Similar increases were seen from preoperative levels to 4 h of reperfusion (0·43 *versus* 0·57 nmol per mg protein; $P = 0{\cdot}001$ and 0·43 *versus* 0·56 nmol per mg protein; $P = 0{\cdot}002$ respectively). NT concentrations returned to preoperative values by 24 h after reperfusion. In contrast, there were no significant perioperative changes in NT levels during endovascular repair for the equivalent time periods.

Conclusion: This study indicated that open repair led to an increased production of reactive nitrogen species compared with endovascular repair. This might explain the greater severity of reperfusion-related events after open repair.

Do POSSUM and P-POSSUM-based models accurately predict mortality following aortic aneurysm repair?

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Background: The aim of this study was to test the predictive accuracy of Physiological and Operative Severity Scores for the enUmeration of Mortality and morbidity (POSSUM) and P-POSSUM models for various modes of presentation of abdominal aortic aneurysm (AAA).

Methods: Some 470 non-consecutive AAAs (297 elective, 70 urgent, 32 stable ruptured and 71 ruptured shocked) performed by five vascular surgeons from 1992 to 2000 were analysed. The mean age was 72 (range 43–87) years and 83 per cent of the patients were men. The physiological and operative parameters and 30-day mortality were collected on preprinted sheets and entered into a database. Patients were stratified into risk groups based on the predicted mortality calculated using the relevant equations. The predicted and the observed deaths were compared with χ^2 analysis.

Results: The observed 30-day mortality rates for elective, urgent, stable ruptured and ruptured shocked categories were 6 per cent (18 of 297), 10 per cent (seven of 70), 25 per cent (eight of 32) and 21 per cent (15 of 71) respectively. The POSSUM model significantly overpredicted mortality ($P < 0{\cdot}001$). Of the P-POSSUM-based models P-POSSUM, V-POSSUM and V-POSSUM (PS = physiology only) models accurately predicted outcome for elective and urgent categories ($P > 0{\cdot}15$). The V-POSSUM model and the ruptured AAA model accurately predicted outcome for ruptured stable AAAs ($P > 0{\cdot}05$), while none of the models accurately predicted mortality for the ruptured shocked AAAs ($P < 0{\cdot}05$).

Conclusion: P-POSSUM-based models accurately predicted outcome for elective, urgent and ruptured stable AAA repairs. Further study on a large series is needed to verify the validity of the current models for ruptured shocked AAA repairs.

Plasma nitrotyrosine: a novel marker of vascular inflammation in abdominal aortic aneurysmal disease?

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Background: Vascular inflammation is implicated in the pathogenesis of atherosclerosis and abdominal aortic aneurysm (AAA), and is thought to involve reactive species such as the nitric oxide-derived oxidant, peroxynitrite. Nitrotyrosine was measured as a stable marker of peroxynitrite production *in vivo*.

Methods: Blood was obtained from patients with an AAA (diameter greater than 5·5 cm; $n = 24$), intermittent claudication (IC; $n = 10$), smoking age-matched controls (SCs; $n = 10$), non-smoking age-matched controls (NSCs; $n = 10$) and non-smoking young healthy controls (YHCs; $n = 10$). Control groups were asymptomatic for peripheral vascular disease. Plasma nitrotyrosine concentrations were measured by an enzyme-linked immunosorbent assay, and expressed as nanomoles per milligram of protein.

Results: The median plasma nitrotyrosine concentration in patients with an AAA was significantly higher than that in patients with IC (0·46 *versus* 0·34 nmol, $P = 0{\cdot}007$), SCs (0·46 *versus* 0·36 nmol, $P = 0{\cdot}007$), NSCs (0·46 *versus* 0·36 nmol, $P = 0{\cdot}005$) and YHCs (0·46 *versus* 0·28 nmol, $P < 0{\cdot}001$). Although there was no significant difference between the IC, SC and NSC groups, all had significantly greater levels than YHCs (0·34 *versus* 0·28 nmol, $P = 0{\cdot}007$; 0·36 *versus* 0·28 nmol, $P = 0{\cdot}04$; 0·36 *versus* 0·28 nmol, $P = 0{\cdot}004$ respectively).

Conclusion: Patients with an AAA had raised levels of circulating nitrated proteins compared with claudication and control groups, suggesting a greater degree of ongoing inflammation that is not related to smoking. Modulation of this inflammatory process may assist in halting the progression of AAA.

Taurine attenuates microvascular injury induced by ischaemia–reperfusion

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Background: Leucocyte adhesion and migration plays a central role in microvascular injury during ischaemia–reperfusion (IR). It was hypothesized that taurine, a semi-essential amino acid and antioxidant, attenuates IR-induced microvascular injury by inhibiting leucocyte–endothelial interactions.

Methods: Sprague–Dawley rats were randomized into control, IR and taurine plus IR groups. IR injury was established by occlusion of the superior mesenteric and coeliac vascular pedicle for 20 min, followed by 60 min of reperfusion. Taurine (200 mg per kg body-weight as a bolus) was administered before IR. The number of adherent and migrated leucocytes, and their rolling velocity, were measured by intravital microscopy in a postcapillary venule.

Results: The *Table* shows the values at 60 min reperfusion.

	Control	IR	Taurine + IR
Rolling velocity (µm s^{-1})	53·4(2·8)*	29·1(3·8)	55·4(6·4)*
No. adhering (per 100 µm)	3·3(1·6)*	25·2(2·2)	4·2(0·7)*
No. migrating (per field)	3·2(2·4)*	11·5(1·6)	3·8(1·1)*

Values are mean(s.e.m.). *$P < 0{\cdot}01$ *versus* IR (analysis of variance with Scheffe *post hoc* test)

Conclusion: These data demonstrate that taurine is effective in inhibiting leucocyte adhesion and migration after IR. The mechanism underlying this protection warrants further investigation.

Regulation of interleukin 6 production by abdominal aortic aneurysms

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Background: Interleukin (IL) 6 is a novel cardiovascular risk factor that is secreted by the abdominal aortic aneurysm (AAA) wall when placed in explant culture. This study investigated whether AAA wall secretes IL-6 *in vivo* and whether prostaglandin (PG) E_2 regulates IL-6 secretion *in vitro*.

Methods: In 19 patients undergoing open AAA surgery, IL-6 levels were measured by enzyme-linked immunosorbent assay in plasma from simultaneous brachial and iliac artery blood samples. The effect of PGE_2 and cyclo-oxygenase inhibitors on IL-6 secretion by explants was determined. IL-6 concentrations are reported as median, with analysis of variance for log-transformed data.

Results: IL-6 concentration in iliac plasma was higher than in brachial plasma (mean difference 26·5 pg ml^{-1}). This difference increased with AAA diameter ($P = 0{\cdot}01$). Indomethacin (10 µm) suppressed the secretion of IL-6 by explants ($n = 11$) from 355 to 105 ng ml^{-1} ($P < 0{\cdot}001$). PGE_2 (0·5 µm) partially offset the indomethacin suppression, and IL-6 secretion increased to 165 ng ml^{-1} ($P = 0{\cdot}05$). Dexamethasone (0·1 µm) completely suppressed IL-6 (and PGE_2) secretion from explants ($n = 8$). The re-addition of PGE_2 did not stimulate IL-6 secretion.

Conclusion: Aneurysms secrete the cardiovascular risk factor IL-6 into the circulation, the amount increasing with AAA diameter. *In vitro*, the secretion of IL-6 by the AAA wall is regulated by a metabolite of PGE_2.

Questionnaires alone may be sufficient for the assessment of patients with intermittent claudication

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Background: Traditional use of treadmill walking and the ankle : brachial pressure index (ABPI) to evaluate patients with intermittent claudication may not adequately address the impact of the disease on patients' quality of life. If new measures such as disease-specific quality-of-life questionnaires (DSQs) and the 6-min walking (SMW) test are to be considered, they should be shown to have greater reliability and improve upon these established tests.

Methods: Thirty-seven patients of median age 68 (range 50–85) years with confirmed peripheral arterial disease were recruited. Each patient was assessed with the validated DSQ, ABPI, constant load treadmill (CLT) test at

3·5 km h^{-1} and 12 per cent gradient to absolute claudication time (ACT), and the SMW test. The CLT and SMW tests were repeated 1 week later to assess reproducibility.

Results: All patients were able to perform the SMW test (mean 392 m) and the DSQ. SMW test variability was 5·4 (95 per cent confidence interval (c.i.) − 9 to 17) per cent. Eighty-nine per cent of patients were able to perform the ACT (mean 127 s). Variability was 23·8 (95 per cent c.i. − 63 to 66) per cent. Spearman correlation was greatest between the DSQ and the SMW test ($r_s = 0{\cdot}57$, $P < 0{\cdot}001$). Other significant correlations were: SMW test with CLT ($r_s = 0{\cdot}46$, $P = 0{\cdot}007$), DSQ with CLT test ($r_s = 0{\cdot}36$, $P = 0{\cdot}04$) and SMW test with ABPI ($r_s = 0{\cdot}32$, $P = 0{\cdot}05$).

Conclusion: The SMW test was easier for patients to perform, more reproducible than the CLT test, and correlated with the DSQ, CLT test and ABPI. The strong correlation between the SMW test and the DSQ means that use of the questionnaire alone may be sufficient to assess and monitor patients with claudication.

Taurine protects against hypoxia–reoxygenation-induced human endothelial apoptosis and necrosis

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Background: Endothelial cell (EC) apoptosis plays an important role in vascular diseases. Hypoxia–reoxygenation (H-R) may induce EC apoptosis and necrosis. This study was designed to investigate the effect of taurine, a semi-essential amino acid and an antioxidant, on H-R-induced EC apoptosis and necrosis.

Methods: Human saphenous vein endothelial cells (HSVECs) were divided into the following groups: control, H-R only, and treatment with taurine (0·5 mg ml^{-1}) before or after hypoxia. Hypoxia was induced by placing HSVECs in a hypoxic chamber for 48 h, followed by reoxygenation under normoxia for 24 h. EC survival, apoptosis and necrosis were measured by flow cytometry. Expression of endothelial nitric oxide synthase (eNOS) of HSVECs was determined by Western immunoblotting.

Results:

	Control	H-R	Taurine + H-R	H–taurine + R
Survival (%)	73·5(1·1)*	49·4(2·8)	71·6(2·9)*	64·7(4·0)*
Apoptosis (%)	14·7(1·7)*	28·5(3·6)	14·8(1·7)*	17·7(1·6)†
Necrosis (%)	8·5(0·8)*	18·2(1·8)	9·7(1·5)*	11·2(1·3)†

Values are mean(s.e.m.). *$P < 0{\cdot}01$ *versus* H-R; †$P < 0{\cdot}05$ *versus* H-R (analysis of variance with Scheffe *post hoc* test)

Expression of eNOS in HSVECs was increased by addition of taurine.

Conclusion: These data demonstrated that taurine protected against H-R-induced endothelial cell apoptosis and necrosis, possibly via upregulation of eNOS.

Prospective randomized trial of the antiendotoxin agent taurolidine in coronary artery bypass surgery

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Background: Cardiopulmonary bypass results in ischaemia–reperfusion-induced endotoxaemia. A prospective randomized trial was performed to investigate the effect of taurolidine, an antiendotoxin agent with antioxidant and membrane-stabilizing properties, on patients undergoing coronary artery bypass surgery (CABS).

Methods: Sixty patients undergoing elective CABS were randomized into four groups. Two groups (1 and 2) received taurolidine intravenously at induction, with two more doses at 12-hour intervals. Groups 3 and 4 received a placebo infusion of normal saline. In groups 1 and 3 St Thomas' cold crystalloid cardioplegia was used, and in groups 2 and 4 cold blood cardioplegia. Levels of the proinflammatory and anti-inflammatory cytokines, interleukin (IL) 6 and IL-10, and the occurrence of ischaemia–reperfusion-induced arrhythmias were assessed during the operative period.

Results: Administration of taurolidine in patients having crystalloid cardioplegia resulted in significantly reduced circulating IL-6 and increased IL-10 concentrations when compared with those receiving crystalloid plus placebo. Taurolidine treatment also significantly reduced reperfusion-induced arrhythmias ($P < 0{\cdot}003$ *versus* placebo). Serum IL-6 and IL-10 levels at 24 h after aortic unclamping are shown in the *Table*.

	IL-6 (pg ml^{-1})	IL-10 (pg ml^{-1})
Crystalloid cardioplegia + taurolidine	8·0(1·4)*	28·2(9·6)*
Crystalloid + placebo	21·3(3·5)	7·9(3·9)

Values are mean(s.d.). *$P < 0{\cdot}05$

Conclusion: This study demonstrates that taurolidine induces a potent anti-inflammatory tissue response in patients undergoing CABS that is associated with a significant reduction in arrhythmias.

The antiendotoxin agent taurolidine inhibits breast cancer *in vitro* and *in vivo*

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Background: There is evidence that the antiendotoxin agent taurolidine has antitumour effects on human colorectal cancer cell lines. The effects of taurolidine on breast cancer cells were assessed *in vitro* and *in vivo*.

Methods: In experiment 1, the metastatic human breast cell line MDA and the murine 4T1 breast cell line were incubated with taurolidine at 5, 10, 25, 50 and 100 μg ml^{-1}. Cell proliferation, apoptosis, cell cycle and lactate dehydrogenase (LDH) release were assessed. In the second experiment, Balb/c mice received tail vein injection of 200 μl 4T1 (5×10^5 per ml) and a subsequent injection of 400 μl taurolidine or phosphate-buffered saline (PBS) every second day, up to day 10. The animals were killed on day 10 and the following assessments were performed: (a) gross metastatic score and (b) histological assessment by a grid mechanism.

Results: Taurolidine (50 and 100 μg ml^{-1}) significantly inhibited tumour cell proliferation at 24 h by approximately 60 and 90 per cent respectively ($P < 0{\cdot}001$). Taurolidine did not alter apoptosis or the phases of the cell cycle. There was a direct cytotoxic effect on the cells, as indicated by greater LDH release (159 and 138 per cent of control with the 100 μg ml^{-1} taurolidine dose in 4T1 and MDA cell lines respectively). The gross metastatic score was 0·9 in the taurolidine group *versus* 3·4 in the PBS group ($P < 0{\cdot}05$). The percentage of lung metastases was 4 per cent in the taurolidine group compared with 11 per cent in the PBS group ($P < 0{\cdot}05$).

Conclusion: Taurolidine inhibited the proliferation of these breast cancer cell lines *in vitro* at subtherapeutic doses, and it also significantly decreased the number of lung metastases in this model. These findings may advocate a role for taurolidine as a further option in treating patients with breast cancer.

Lipopolysaccharide pretreatment reduces nuclear factor κB transcriptional activity and tumour cell adhesion in response to further stimulation by lipopolysaccharide

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Backround: Endotoxaemia at the time of surgery has been linked to postoperative tumour progression. It was hypothesized that pretreatment of tumour cells with low-dose endotoxin would alter the lipopolysaccharide (LPS)-induced activation of nuclear factor (NF) κB compared with that in non-pretreated cells. This action would reduce tumour cell adhesiveness, which is a NF-κB-dependent process.

Methods: SW480 colonic tumour cells were pretreated with low-dose LPS (10 ng ml^{-1}) for 24 h and then stimulated with LPS (100 ng ml^{-1}), or medium as control. NF-κB translocation and its subunit composition was assessed using electrophoretic mobility shift assay and supershift analysis. Transcriptional activation was assessed by transfecting cells with the pNF-κB-luc vector. Tumour cell adhesion to fibronectin was assessed by means of the Cell One Solution assay. Results are given as mean(s.e.m.) in relative light units (RLU). Statistical analysis was by analysis of variance.

Results: Stimulation of pretreated cells resulted in earlier and increased NF-κB translocation compared with that in naive cells. NF-κB subunit composition differed in pretreated cells with an increase in the inhibitory p50/p50 homodimer following stimulation with LPS. Reporter plasmid transfection showed that stimulation of non-pretreated cells with LPS resulted in increased transcriptional activity (0·32(0·04) *versus* 0·67(0·11) RLU; $P < 0{\cdot}01$) and increased adherence to fibronectin (0·29(0·02) *versus* 0·39(0·02) RLU; $P = 0{\cdot}04$). Transcriptional activity was not increased in stimulated pretreated cells (0·31(0·05) *versus* 0·33(0·03) RLU; $P > 0{\cdot}05$) and cell adhesion was not significantly increased (0·35(0·05) *versus* 0·36(0·05) RLU; $P = 0{\cdot}9$).

Conclusion: Pretreatment of SW480 tumour cells with low-dose LPS reduces the ability of high-dose LPS to induce NF-κB transcriptional activity and to promote tumour cell adhesion. The results suggest a novel therapeutic strategy for the prevention of postoperative tumour metastasis.

Regulation of human liver microvascular endothelial cell apoptosis by a tyrosine phosphatase inhibitor

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Background: Human liver microvascular endothelial cells (HLMVECs) undergo rapid apoptosis when cultured in a serum- and growth factor-free medium. The tyrosine phosphatase inhibitor, sodium metavanadate, is a potent endothelial cell protector even against potent apoptosis-inducing agents such as endostatin and tumour necrosis factor (TNF) α. Because the process of phosphorylation and dephosphorylation is vital in the endothelial cell apoptosis pathway, the role tyrosine phosphorylation in HLMVECs cultured in a serum- and growth factor-free medium was examined.

Methods: HLMVECs were grown in complete serum-free media (with endothelial growth factors) at 37°C in 5 per cent carbon dioxide. Some 1×10^4 cells were incubated per well. Cells were starved with 2 per cent fetal calf serum for 24 h and subsequently incubated with sodium metavanadate (5 and 10 μmol l^{-1}), vascular endothelial growth factor (VEGF; 5 ng ml^{-1}) and Genistein (5, 10 and 20 μmol l^{-1}) for a further 48 h. Negative controls were treated with phosphate-buffered saline. Apoptosis was assessed by enzyme-linked immunosorbent assay, which measures intracellular nucleosomes. Each experiment was done in triplicate and repeated three times.

Results: Treatment of the cells with metavanadate decreased the apoptotic index (AI) to 0·6 at a dose of 5 μmol l^{-1} and to 0·6 at 10 μmol l^{-1}. The VEGF group showed a marginal decrease in apoptosis (AI 0·9), but in the presence of metavanadate the AI was further reduced to 0·3. Genistein increased the AI to 1·2.

Conclusion: HLMVECs undergo apoptosis in the absence of adequate serum or growth factors, and are protected by the tyrosine phosphatase inhibitor, sodium metavanadate. This suggests that activation of tyrosine phosphatase may initiate apoptosis in HLMVECs that are cultured in media depleted of serum and growth factors.

Endostatin is released from human platelets

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Background: Endostatin is an endogenous angiogenesis inhibitor demonstrated to reduce tumour growth and metastases *in vivo*. Although its source is unclear, there is some evidence from animal studies suggesting platelet storage. It was hypothesized that, if endostatin were stored in platelets and released on platelet activation, endostatin concentration in serum would be higher than that in plasma.

Methods: The serum and plasma of 21 patients with colorectal tumours were compared with those from 40 age-matched healthy subjects, using a standard enzyme-linked immunosorbent assay to measure endostatin levels and correlating these with individual platelet counts. The effect of thrombin-induced platelet aggregation on endostatin release from pooled platelets obtained from the national blood bank was also determined. Platelet concentrates were stimulated with 1 unit thrombin and, after centrifugation at 5000 r.p.m. for 15 min, were stored at −70°C. Washed platelets were prepared as described previously and compared with platelet-rich plasma (PRP). Each experiment was conducted at a concentration of 1×10^8 cells per ml.

Results: There was no significant difference between endostatin levels in serum and those in plasma in the 21 patients with colorectal cancer ($P = 0{\cdot}15$). The level of endostatin in serum was significantly lower in the cancer group than in controls (53·0 (interquartile range 38·2–74·4) *versus* 86·0 (44·9–131·0) ng ml^{-1}; $P = 0{\cdot}001$). The platelet count was significantly higher in the cancer group compared with the control group (324 10^9/L (250–478) *versus* 229 (179–269); $P = 0{\cdot}001$). There was no correlation between endostatin level and platelet counts in either cancer ($r = 0{\cdot}32$, $P = 0{\cdot}15$) or control ($r = -0{\cdot}15$, $P = 0{\cdot}36$) groups. There was a threefold increase in endostatin on stimulating pooled PRP (20·6 *versus* 60·4 ng ml^{-1}; $P < 0{\cdot}001$) with 1 unit thrombin. Washed platelets were found to release a mean(s.d.) of 1·5(0·4) ng ml^{-1} endostatin on the addition of a similar concentration of thrombin. The washed platelet lysate contained 4·4(1·6) ng ml^{-1} endostatin.

Conclusion: These data suggest that human platelets release endostatin on thrombin stimulation. The results also support the hypothesis that platelet activation may have an important contribution to angiogenic balance.

The antiendotoxin agent taurolidine inhibits angiogenesis

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Background: The development of metastases in patients with cancer is known to be dependent on angiogenesis. Taurolidine is an antiendotoxin agent, and also has antineoplastic effects. Taurolidine may also inhibit angiogenesis.

Methods: Human umbilical vein endothelial cells (HUVECs) were treated with taurolidine and cell proliferation was assessed using 5-bromo-2-deoxyuridine incorporation. Capillary tube formation following treatment with taurolidine, with and without stimulation with vascular endothelial growth factor (VEGF) was assessed on matrigel. Aortic ring sprouting using aortas from 3–4-week-old rats was also examined for inhibition after incubation with taurolidine.

Results: The results for HUVEC proliferation, capillary tubule formation and aortic ring sprouting are shown in the *Table*. Taurolidine completely inhibited capillary tubule formation at doses of 50, 100 and 200 µg ml^{-1}. Taurolidine also inhibited VEGF-stimulated capillary tubule formation at concentrations of 5, 25, 50 and 100 µg ml^{-1}, decreasing mean(s.e.m.) values from 58(2) to 42(2), 8(3), 3(1) and 0(0) respectively. Aortic ring sprouting was completely inhibited at 100 and 200 µg ml^{-1} doses of taurolidine.

		Taurolidine (µg ml^{-1})				
	Control	5	25	50	100	200
HUVEC proliferation (%)	100	99(5)	19(4)*	9(3)*	10(2)*	10(1)
Capillary tubule formation (no.)	44	32(4)	6(4)*	0(0)*	0(0)*	0(0)*
Aortic ring sprouting (%)	100	—	34(15)*	6(5)*	0(0)*	0(0)*

*$P < 0{\cdot}05$ *versus* control (analysis of variance)

Conclusion: These findings demonstrate that taurolidine inhibits angiogenesis in a dose-dependent manner. Taurolidine may prevent VEGF-mediated angiogenesis and thus may potentially inhibit the growth of dormant micrometastases following surgical removal of a primary tumour.

Reduction of c-*myc* proto-oncogene expression in cutaneous melanoma

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Background: The c-*myc* proto-oncogene plays a key role in cell proliferation, malignant transformation and apoptosis. Previous work demonstrated that c-*myc* expression levels are an accurate prognostic marker in melanoma and c-*myc* is therefore regarded as a potential target for corrective gene therapy.

Methods: Antisense oligonucleotides complementary to the c-*myc* translation initiation site were constructed. Immunodeficient mice were then implanted with A375M cells and treated with antisense oligoncleotides. A ribozyme was constructed targeting the same region of messenger RNA as the antisense. A375M melanoma cells were transfected using liposomes, and growth was assessed with a methyltetrazolium sulphate growth assay. C-*myc* expression was measured by means of immunostaining and flow cytometry.

Results: Antisense oligoncleotides reduced the growth of A375M cells *in vitro* and this reduction could be correlated with decreased c-*myc* gene activity. Tumours in mice had developed in 21 per cent of the treated group compared to 73 per cent in the group treated with a scrambled oligonucleotide and 100 per cent in the saline treated controls. The ribozyme did not reduce growth or c-*myc* expression. Further ribozymes targeting different regions of the c-*myc* gene are being assessed.

Conclusion: Oligonucleotides are an effective method of reducing c-*myc* expression *in vitro* and *in vivo*. Development of ribozymes may provide a useful new gene therapy for the treatment of melanoma.

Hypertonic saline impedes tumour cell–endothelial cell interaction by inhibiting adhesion molecule and laminin expression

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Background: Haematogenous cancer spread is initiated by the adherence of tumour cells to the vascular endothelium, enhanced by perioperative endotoxin (lipopolysaccharide; LPS) and endogenous inflammatory mediators. This study tested the hypothesis that hypertonic saline (HTS) attenuates tumour cell adhesion to the endothelium by reducing adhesion molecule expression.

Methods: Human colonic cancer cells (LS174T) were transfected with green fluorescent protein and exposed to LPS, tumour necrosis factor (TNF) α and interleukin (IL) 6 under hypertonic (Na^+ 180 mmol l^{-1}) and normotonic (Na^+ 140 mmol l^{-1}) conditions for 1 and 4 h. Confluent human umbilical vein endothelial cells (HUVECs) were similarly exposed. Cellular apoptosis and expression of adhesion molecules (α_1-integrin and intercellular adhesion molecule (ICAM) 1) and laminin were measured by flow cytometry. Tumour cell adhesion to endothelial cells, laminin and fibronectin was assessed with fluorescence microscopy.

Results: Hypertonic exposure significantly reduced tumour cell adhesion, even in the presence of the perioperative cell stressors (*Table*). HTS attenuated tumour cell α_1-integrin and ICAM-1 expression, as well as endothelial cell ICAM-1 and laminin expression ($P < 0{\cdot}04$), but did not significantly alter cell viability (*Table*).

	Control	HTS	LPS + HTS
Adhesion to HUVECs (cell count)*	119(22)	10(2)†	24(3)†
Adhesion to laminin (cell count)*	125(23)	14(2)†	20(3)†
α_1-Integrin expression (MCF)	23·8	12·9†	14·4†
Apoptosis rate in 24 h (%)	35·4	34·5	30·6

*Values are mean(s.e.m.). MCF, mean channel fluorescence. †$P < 0{\cdot}04$ *versus* control (analysis of variance)

Conclusion: HTS significantly attenuates tumour cell adhesion to the endothelium by inhibiting adhesion molecule and laminin expression. This may provide a strategy to halt the metastatic behaviour of tumour cells shed at operation by preventing endothelial adherence.

Chromosomal abnormality and gene mutation in sporadic colorectal cancer

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Background: During recent years the molecular genetic approach to colorectal tumourgenesis has been favoured but there is increasing evidence that characterization of chromosomal abnormalities may be of fundamental importance. This study was designed to ascertain patterns of chromosomal abnormality in a relatively large series of colorectal cancers and compare their frequency with the frequency of common gene mutations.

Methods: Fifty primary colorectal cancers were analysed for chromosomal abnormalities using comparative genomic hybridization (CGH). These tumours were randomly selected from a cohort of 106 cases for which the mutational status of the genes *APC*, K-*ras* and *p53*, and the immunohistochemical pattern for the mismatch repair proteins human mutL homologue 1 (hMLH1) and human mutS homologue 2 (hMSH2), had been ascertained.

Results: The mean number of chromosomal aberrations per tumour was 7·5 (range 0–28). The single most frequent genetic abnormality was gain of chromosome arm 20q, which occurred in 74 per cent of tumours. Other common chromosomal aberrations were gain of 13q, 8q and 7p, and loss of 18q, which affected 58, 46, 46 and 46 per cent of cases respectively. Mutation of *p53*, *APC* and K-*ras* was found in 61, 56 and 27 per cent of tumours respectively.

Conclusion: The most striking finding from this study was that the single most frequent genetic aberration in these colorectal cancers was chromosomal and involved a region in which there is no convincing candidate gene currently known to be involved in colorectal tumorigenesis. Furthermore, the results reinforce the utility of CGH as a powerful, unbiased, genetic screen which can be used to search for novel genes involved in tumourgenesis.

Cyclo-oxygenase 2: a possible target in schistosome-associated bladder cancer

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Background: Recent evidence suggests that non-steroidal anti-inflammatory drugs exert their antitumour effect via inhibition of cyclo-oxygenase (COX) 1 and COX-2. These enzymes and their products, prostaglandins, are thought to play a role in the progression of many human cancers. Their role in schistosome-associated bladder cancer (SABC) remains to be defined.

Methods: Sixty paired tumour and normal samples of SABC were assessed for the expression of COX-1 and COX-2 by immunohistochemistry. Results were correlated with stage and grade.

Results: COX-1 was expressed ubiquitously, while there was a significant increase in COX-2 ($P < 0·001$) with moderate to strong granular cytoplasmic expression in transitional cell carcinoma (TCC), squamous cell carcinoma (SCC) and adenocarcinomas associated with schistosomiasis, compared with non-malignant urothelium. COX-2 reactivity was higher in TCCs and adenocarcinomas compared to SCCs ($P < 0·001$). Areas of carcinoma *in situ* showed COX-2 reactivity comparable to that observed in invasive areas. A statistically significant positive correlation was found between COX-2 expression and tumour grade ($P = 0·005$).

Conclusion: COX-2 is overexpressed in SABC, consistent with a potential role for COX-2 inhibitors in the prevention and management of SABC.

Bacterial lipoprotein and lipopolysaccharide tolerance in macrophages is mediated by interleukin 1 receptor-associated kinase phosphorylation

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Background: Interleukin 1 receptor-associated kinase (IRAK) is a signal transducer for proinflammatory cytokines. The role of IRAK in tolerance to lipopolysaccharide (LPS) and bacterial lipoprotein (BLP) was investigated.

Methods: RAW 264·7 macrophages were pretreated with medium, LPS or BLP (10 ng ml^{-1}) for 24 h, then stimulated with LPS, BLP or LPS + BLP (100 and 1000 ng ml^{-1}). Activated IRAK was determined by Western blotting. Next, the macrophages were pretreated with medium, AG126 or AG556, for 1 h and stimulated with LPS, BLP or LPS + BLP at 100- and 1000-ng ml^{-1} doses. Tumour necrosis factor (TNF) α was measured by enzyme-linked immunosorbent assay. To validate the *in vitro* findings, caecal ligation and puncture was performed on CD-1 mice after pretreatment with AG556.

Results: Pretreatment with tyrophostin produced a dose-dependent decrease in TNF-α production in all groups. LPS, BLP and LPS + BLP upregulated IRAK in macrophages. There was a marked downregulation of activated IRAK protein in both BLP- and LPS-tolerant macrophages. Administration of IRAK inhibitors in mice attenuated lethality compared with controls.

	TNF-α production (ng ml^{-1})		
	Medium	AG126	AG556
LPS			
100 ng	20(0·8)	3·0(0·4)*	1·7(0·3)*
1000 ng	22(2·8)	2·7(0·04)*	2·3(0·06)*
BLP			
100 ng	28(0·9)	2·7(0·3)*	4·9(0·7)*
1000 ng	24(0·8)	2·6(0·3)*	3·5(0·1)*
LPS + BLP			
100 ng	30(0·6)	2·3(0·2)*	5·7(0·5)*
1000 ng	28(1·3)	2·2(0·2)*	3·6(0·5)*

Values are mean(s.d.). *$P < 0·001$ *versus* control (analysis of variance)

Conclusion: These results suggest that IRAK serves as a common upstream signal transducer for proinflammatory cytokine production. As inhibition of IRAK activation is intrinsic to BLP and LPS tolerance-induced changes, inhibition may significantly modulate the systemic inflammatory response to sepsis.

Relationship between intestinal permeability to macromolecular probes and postoperative endotoxaemia

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Background: There is evidence to suggest macromolecular intestinal permeability (IP) probes could be of greater value in assessing the gut barrier. The aim of this study was to correlate the alterations in markers of endotoxaemia with changes in intestinal permeability to a macromolecular probe.

Methods: Fifty-one patients undergoing radical gastro-oesophageal resection for malignancy had IP measured before and 7 days after operation using a dual polyethylene glycol (PEG3350) technique. Plasma immunoglobulin M to the anticore endotoxin (IgM EndoCAb) molecule was also measured.

Results: In the 17 patients who developed infective complications, PEG3350 retrieval increased from 0·07 (0·06–0·09) per cent before operation to 0·18 (0·10–0·24) per cent after operation ($P = 0{\cdot}01$). IgM EndoCAb increased from 65 (43–76) to 82 (71–187) MU l^{-1} ($P = 0{\cdot}04$). In the 34 patients who experienced an uncomplicated recovery, PEG3350 retrieval increased from 0·08 (0·06–0·12) per cent before operation to 0·16 (0·14–0·24) per cent ($P = 0{\cdot}007$). IgM EndoCAb remained unchanged: from 72 (37–101) to 82 (54–128) MU l^{-1}. There were no significant differences between the two groups in either PEG retrieval or IgM EndoCAb titre. There was no correlation between PEG3350 retrieval and IgM EndoCAb titre ($r_s = -0{\cdot}003$, $P = 0{\cdot}82$).

Conclusion: There does not appear to be a correlation between absorption of PEG3350 and evidence of endotoxaemia. These findings may be explained by different mechanisms of absorption of the two molecules (transcellular *versus* paracellular), and call into question the value of orally ingested IP probes to assess gut barrier function.

Characterization of hepatic proinflammatory and anti-inflammatory cytokine responses to portal endotoxaemia in obstructive jaundice

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Background: Alteration in hepatic proinflammatory and anti-inflammatory cytokine responses to portal endotoxaemia may contribute to the observed organ dysfunction associated with intervention in obstructive jaundice. This study investigated the effect of obstructive jaundice on the liver's proinflammatory cytokines, tumour necrosis factor (TNF) α and interleukin (IL) 6, and the anti-inflammatory cytokine IL-10, in response to portal endotoxaemia.

Methods: Male Wistar rats (250–350 g) were allocated randomly to either bile duct ligation (BDL) ($n = 8$) or sham operation ($n = 8$). One week after surgery they underwent isolated hepatic perfusion. Endotoxin (*Escherichia coli* 0111:B4) was added to the perfusate from time 10–20 min. Aliquots of effluent perfusate were collected for cytokine analysis at 80 and 100 min after endotoxin perfusion.

Results:

	Time after endotoxin perfusion (min)	
	80	100
TNF-α (pg ml^{-1})		
BDL	3594 (1243–6696)*	3566 (1212–8398)*
Sham	421 (240–709)	363 (238–578)
IL-6 (pg ml^{-1})		
BDL	247 (158–692)*	1168 (668–2382)*
Sham	0 (0–5)	0 (0–42)
IL-10 (pg ml^{-1})		
BDL	8 (5–17)	11 (7–26)*
Sham	5 (4–9)	7 (5–8)

Values are median (interquartile range). $^*P < 0{\cdot}05$ *versus* sham (Mann–Whitney *U* test)

Conclusion: Endotoxaemia in obstructive jaundice results in a significant increase in the proinflammatory and anti-inflammatory cytokine response. The exaggerated anti-inflammatory response may contribute to immune depression, thus providing an environment conducive to subsequent sepsis and organ dysfunction.

Bacterial lipoprotein activates nuclear factor κB and delays neutrophil apoptosis via a pathway involving p38 mitogen-activated protein kinase and phosphatidylinositol 3′-kinase

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Background: Delayed neutrophil apoptosis is implicated in the pathophysiology of septic shock. Bacterial lipoprotein (BLP) is a ubiquitous component of both Gram-positive and Gram-negative bacteria. This study examined the effect of BLP on neutrophil activation, particularly nuclear factor (NF) κB activation, and the mechanism by which this bacterial wall product delays spontaneous neutrophil apoptosis.

Methods: Neutrophils isolated from healthy donors were incubated with BLP (100 g ml^{-1}) and apoptosis was assessed at 12 and 24 h by annexin V binding measured by flow cytometry. Nuclear extracts were assessed for NF-κB activity by electrophoretic mobility shift analysis. Supershift analysis was performed with specific antibodies to the p50 and p65 subunits. Cells were also preincubated with specific inhibitors to NF-κB (SN50 50 μmol l^{-1}), p38 mitogen-activated protein (MAP) kinase (SB202190 25 μmol l^{-1}), Jun N-terminal kinase (JNK) (Dicumarol 75 μmol l^{-1}) and phosphatidylinositol 3′-kinase (PI-3 kinase) (LY294002 100 μmol l^{-1}), and the effect on BLP-induced apoptosis was assessed.

Results: BLP activated NF-κB, with maximal activity seen at 30 min, and supershift analysis demonstrated an increase in the p65 subunit compared with unstimulated cells. Inhibition of NF-κB or JNK did not affect the BLP-induced decrease in apoptosis which was significantly delayed compared with that in unstimulated cells at 24 h (mean(s.e.m.) 15·5(5·8) *versus* 46·7(9·0) per cent; $P < 0·05$, analysis of variance). Inhibition of PI-3 kinase reduced the BLP-induced apoptotic delay at 24 h (24·6(5·5) *versus* 5·4(1·3) per cent; $P = 0·02$), which was still less than the level of apoptosis in control cells (64·0(0·1 per cent). Inhibition of p38 MAP kinase also reduced the BLP-induced apoptotic delay assessed at 24 h to within normal limits (44·7(7·1) *versus* 12·4(5·5) per cent; $P = 0·03$).

Conclusion: BLP activates NF-κB in human neutrophils and delays apoptosis through a pathway involving p38 MAP kinase and PI-3 kinase.

Identification of a potential new antiscarring agent

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Background: Myofibroblasts are known to persist well beyond the proliferative phase of wound healing in pathological scars, suggesting that they contribute towards the aetiology. A polypeptide factor has been identified in serum which inhibits the formation of myofibroblasts and is thus of potential therapeutic use. The results of its application both *in vitro* and to an incisional wound model are presented.

Methods: Cultured fibroblasts (dermal, normal and pathological scars) were routinely grown in 10 per cent serum-containing media. Cells were then treated for 14 days with medium supplemented with variously treated sera or with insulin-like growth factor (IGF) I and IGF-II, or with insulin. Serum was depleted of active growth factors, including transforming growth factor (TGF) β, using reducing agents and was used to constitute 'growth factor-depleted medium'. Serum was also fractionated according to molecular weight using dialysis and ultrafiltration. The cells were then stained immunohistochemically for α-smooth muscle actin (a myofibroblast marker) and the proportion of myofibroblasts was assessed. *In vivo* effects on wound healing were assessed using two 1·5-cm full-thickness wounds on the posterior flanks of Balb/c mice. One wound was treated with insulin at the time of wounding and the other was treated with saline as control. Wounds were harvested at 2 weeks, processed for histological examination and stained for myofibroblasts.

Results: Culture of all fibroblast types in 'growth factor-depleted medium' resulted in a significant increase in myofibroblasts (24–40 per cent; $P < 0·01$), implying the presence in serum of a polypeptide inhibitor of myofibroblasts. The size of this factor was less than 10 kDa. Of the factors less than 10 kDa commonly found in serum, insulin but not IGF-I or IGF-II was found to inhibit myofibroblast differentiation *in vitro* ($C_{50} = 20–50\ \text{ng ml}^{-1}$; $P < 0·01$). *In vivo*, topical administration of insulin significantly reduced the numbers of myofibroblasts. Optimal results were achieved by a single application of insulin given at the time of wounding.

Conclusion: Insulin reduces myofibroblast formation in fibroblast cultures both *in vitro* and in an *in vivo* wound-healing model. This suggests that insulin may form the basis of a novel and effective antiscarring therapy.

Early local vascular endothelial growth factor release, facilitated by mast cells, may mediate adhesion formation after laparotomy

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Background: Mast cells may initiate the peritoneal reaction to injury, which leads to adhesion formation. A vascular endothelial growth factor (VEGF)-mediated pathway for this process may be stimulated by bacterial antigens.

Methods: Two groups of CD-1 mice underwent standardized adhesion-inducing surgery (AIS), receiving either a single postoperative dose of anti-VEGF monoclonal antibody (10 μg per mouse) or a similar volume of immunoglobulin G isotype antibody. Mice were graded blindly for adhesion formation on a 0–3 scale 14 days later. VEGF was measured by enzyme-linked immunosorbent assay in both systemic plasma and peritoneal lavage fluid. The fluid was obtained 2, 6, 12 and 24 h after surgery in additional CD-1 mice, as well as from a mast cell-deficient strain ($WBB6F1^-$) and their wild-type littermates ($WBB6F1^+$). VEGF was also assessed in unoperated CD-1 mice 2 and 6 h after intraperitoneal administration of either lipopolysaccharide (LPS; 10 or 50 μg per mouse) or vehicle.

Results: CD-1 mice treated with a single dose of anti-VEGF therapy early after surgery had significantly fewer adhesions than controls (mean(s.e.m.) 1·8(0·2) *versus* 2·7(0·2); $P < 0·05$, analysis of variance). Local VEGF release peaked 6 h after surgery in both $WBB6F1^+$ (43(6) pg ml^{-1}) and CD-1 (33(6) pg ml^{-1}) mice compared with controls (18(1) pg ml^{-1}; $P < 0·05$, Mann–Whitney *U* test), but remained similar to baseline in $WBB6F1^-$ mice. Intraperitoneal LPS resulted in significant, dose-dependent, early local release of VEGF in CD-1 mice, while systemic VEGF levels were similar at all times.

Conclusion: These results indicate that mast cell-facilitated local VEGF release plays a central role in the formation of adhesions after laparotomy and may be stimulated by local contamination with bacterial antigens.

Bacterial superantigen enhances wound healing

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Background: Although T cells and their products are known to play a central role in wound healing, the effect of T-cell expansion and activation on wound healing has not been examined. This study examined T-cell activation by bacterial superantigen (staphylococcal enterotoxin B (SEB)) and the effect that this has on wound healing in normal animals.

Methods: Male 10-week-old C57B/L6 mice were treated with SEB 50 mg in 0·5 ml saline or 0·5 ml saline vehicle alone. Serum was harvested at 2 h and cytokine enzyme-linked immunosorbent assay was performed. Two groups of mice were anaesthetized and had a dorsal full-thickness wound (1·5 cm^2) excised, covered with transparent adherent dressing, and treated topically with 0·5 ml saline vehicle or SEB (10 μg). Two groups had a longitudinal dorsal incision through skin and panniculus carnosus, closed with staples and infiltrated with either saline or SEB. Wounds were treated on alternate days and assessed by planimetry for closure. Tensiometry was performed on day 11.

Results: SEB-treated mice produced a proinflammatory T-cell phenotype. Levels of the cytokines interleukin 2 (38 units ml^{-1}), tumour necrosis factor α (810 pg ml^{-1}) and interferon γ (30 ng ml^{-1}) were increased compared with controls ($P < 0·05$, analysis of variance). Planimetry with reference to the original wound area demonstrated enhanced contraction of SEB-treated wounds compared with saline alone wounds (79 *versus* 94 per cent at day 11; $P = 0·02$, Mann–Whitney *U* test). No difference was found in wound tensiometry parameters between SEB and saline groups (1·12 *versus* 1·08 N m^{-2}; $P > 0·05$).

Conclusion: Taken together, these results suggest that T-cell activation with superantigen may enhance wound closure without compromising wound strength. It is postulated that T-cell activation using superantigen may be of therapeutic use to enhance wound healing.

$CD14^+$–$CD16^+$ blood monocyte subset and not genetic predisposition influences the soluble CD14 receptor expression associated with severe acute pancreatitis

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Background: The soluble form of CD14 (sCD14) is derived from a 55-kDa membrane-bound glycoprotein on monocytes and enhances endothelial cytokine responses to lipopolysaccharide (LPS). It is a mediator of the systemic inflammatory response syndrome (SIRS). The role of sCD14 expression in the SIRS associated with acute pancreatitis (AP) was investigated to determine whether altered expression was due to a C260T polymorphism in the CD14 promoter gene or attributable to an altered monocyte subpopulation.

Methods: Peripheral blood samples in patients with AP were assayed for sCD14 (24 and 72 h from onset) and correlated with clinical severity (Atlanta criteria) and SIRS (Acute Physiology Score; APS). Peripheral blood mononuclear cells (PBMCs) were isolated to identify immunophenotypes using immunofluorescence flow cytometry. Leucocyte DNA was genotyped for the CD14 polymorphism using the polymerase chain reaction.

Results: Severe AP ($n = 20$) was associated with an early sustained increase in plasma levels of sCD14 (median 67 (range 25–216) μg l^{-1}) compared with the level in mild attacks (50 (24–103) μg l^{-1}; $n = 70$, $P < 0·001$) and in healthy controls (51 (23–78) μg l^{-1}; $n = 40$, $P < 0·001$). Soluble CD14 correlated strongly with APS at 24 h ($r^2 = 0·38$, $P < 0·001$) and 72 h ($r^2 = 0·56$, $P < 0·001$). The proportion of monocytes in PBMC isolates was increased in severe attacks ($P < 0·05$), but the early increase in CD14 correlated only with the relative expansion in the population of $CD14^+$–$CD16^+$ monocytes ($r^2 = 0·57$, $P < 0·001$). No differences in CD14 genotype were detected between 245 patients with AP (68 severe) and 143 controls, even after stratification for disease severity.

Conclusion: Severe AP is associated with increased sCD14 expression which may be secondary to alterations in monocyte subsets. This increased expression is possibly a response to LPS but does not appear to be predetermined genetically.

Oestrogen withdrawal reduces cell proliferation only in oestrogen receptor-positive ductal carcinoma *in situ*

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Background: In the USA, adjuvant tamoxifen is recommended for all patients following breast-conserving surgery for ductal carcinoma *in situ* (DCIS) despite 50 per cent of cases of DCIS being oestrogen receptor (ER) negative and therefore unlikely to respond to antioestrogen therapy.

Methods: To investigate the effect of oestrogen withdrawal on cell proliferation in patients with DCIS, 111 patients were studied who had undergone diagnostic core biopsy followed by surgery for DCIS 14–21 days later. ER status and Ki67 (a measure of epithelial cell proliferation) were determined for each patient by immunohistochemistry on paired sections of the core biopsy and operative specimens. Women had either never taken, continued or stopped hormone replacement therapy between samples.

Results: The results are summarized in the *Table*.

ER status	n	Median core Ki67	Operative Ki67	P*
Never taken HRT (control)				
Negative	24	9·8 (5·6–18·5)	11·3 (7·5–15·5)	0·92
Positive	32	8·2 (4·9–14·9)	7·7 (4·4–11·8)	0·49
Continued HRT (control)				
Negative	6	7·1 (2·9–18·2)	8·8 (3·9–22·6)	0·94
Positive	19	8·8 (4·3–13·9)	8·8 (2·5–13·6)	0·76
Stopped HRT				
Negative	10	14·4 (9·2–18·9)	16·3 (10·7–23·7)	0·46
Positive	20	8·3 (2·7–16·9)	2·2 (1·07–8·5)	0·03

Values in parentheses are interquartile ranges. *Mann–Whitney U test

Conclusion: Oestrogen withdrawal reduced proliferation in ER-positive but not in ER-negative DCIS. Selection for adjuvant antioestrogen therapy should be based on ER-positive status in patients with DCIS.

Improved detection of circulating tumour cells in patients with breast carcinoma

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Background: There is a variability in the results for the detection of peripheral blood (PB) or bone marrow (BM) circulating tumour cells (CTCs). This unit has validated a novel technique that combines immunomagnetic separation (IMS) with dual-staining flow cytometry (FC), fluorescent microscopy (FM) and immunocytochemistry (ICC). This study aimed to apply this technique to patients with breast carcinoma ($n = 27$), to identify an optimal time and site for sampling.

Methods: PB, rib BM and iliac crest BM samples were taken before and after operation. Axillary vein blood was sampled during resection of the primary tumour. The leucocyte fraction was fixed and permeabilized. Immunostaining of 1×10^6 cells with both fluorescein isothiocyanate (FITC)-conjugated MNF116 (a monoclonal antibody to cytokeratins 5, 6, 8, 17, 18 and 19) and propidium iodide (to differentiate cells from debris) demonstrated specific binding on FC, IMS with FC, and FM. IMS enriched samples by coupling FITC-MNF116^{+} cells to anti-FITC microbeads. An unconjugated form of MNF116 was required for ICC.

Results: Four million cells were evaluated on 124 samples; 88 per cent of patients were positive for CTCs.

Sampling time:

Preop.	Periop.	Postop.
31 of 79 (39%)	8 of 15 (53%)	26 of 54 (46%)

Sampling site:

PB	Axillary blood	Rib BM	Iliac crest BM
15 of 53 (28%)	8 of 15 (53%)	23 of 46 (50%)	26 of 54 (46%)

Technique:

FM/ICC (a)	IMS + FC (b)	(a) and (b)
14%	40%	46%

There was a significant association between positivity rates before and after operation ($P < 0{\cdot}001$, Pearson correlation). There was no significant association between rib and iliac crest BM sampling ($P < 0{\cdot}5$). IMS increased the detection rate from 24 to 40 per cent.

Conclusion: IMS improved detection. This may also be due to multiple sampling at different sites rather than different timepoints. This suggests that phenotyping rather than detection of CTCs may be more important to determine metastatic potential.

Effects of retinoic acid and insulin-like growth factors in primary hyperparathyroidism

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Background: The aim was to assess the inhibitory actions of retinoic acid (RA) and its interactions with the insulin-like growth factor (IGF) system in a primary cell culture model of primary hyperparathryoidism.

Methods: Fresh human parathyroid glands were obtained from nine patients undergoing surgical treatment for primary hyperparathyroidism. Glands were bisected for histology (frozen section) and immediate cell culture. Tissue was dispersed by enzyme digestion and cells were cultured in 24-well plates. Following adhesion, the cells were transferred to serum-free medium and dosed once with growth factors with and without RA for 96 h. Proliferation was assessed by measuring the incorporation of tritiated thymidine.

Results: Compared with controls ($n = 5$), IGF-I and IGF-II at 100 ng ml^{-1} increased DNA synthesis by a mean(s.e.m.) of 56(6) and 51(8) per cent respectively ($P < 0{\cdot}05$). *Trans*-retinoic acid at 10^{-5} mol l^{-1} significantly suppressed basal DNA synthesis by 18(4) per cent, whereas RA at 10^{-6} mol l^{-1} induced DNA synthesis by 16(13) per cent compared with the control ($P < 0{\cdot}05$). RA at 10^{-5} mol l^{-1} completely abrogated the proliferative actions of IGF-II by lowering the thymidine incorporation to 30(10) per cent below the control value ($P < 0{\cdot}05$), but had no significant effect on the IGF-I response. To evaluate the role of IGF-II receptor or IGF-binding proteins (IGFBPs) in mediating the actions of

RA ($n = 4$), the analogues [Leu27]IGF-II (10–20-fold reduced IGF-I receptor affinity) and des(1–6)IGF-II (lower IGFBP binding affinity) were used. The IGF-II inhibitory effect of RA was enhanced in the presence of the analogue [Leu27]IGF-II ($P = 0{\cdot}052$) but not with des(1–6)IGF-II ($P > 0{\cdot}05$) compared with wild-type IGF-II.

Conclusion: These data indicate a novel antiproliferative role for RA in enhancing the pericellular clearance of IGF-II via the IGF-II receptor, thus preventing ligand activation of the IGF-I receptor. This may have broader implications for RA effects in other tumours.

Nitric oxide expression following reperfusion in human orthotopic liver transplantation

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Background: Cold ischaemia followed by reperfusion may cause donor liver injury during liver transplantation (LT). Animal models have suggested that nitric oxide may be an important mediator of this injury. In this study the role of nitric oxide and endothelial and inducible nitric oxide synthases (eNOS and iNOS) in hepatic ischaemia–reperfusion injury during human LT was examined.

Methods: Nine liver samples were obtained from the donor organ before cold perfusion and 1 h after reimplantation. Wedge biopsies (50 mg) were homogenized, centrifuged and filtered (50 000 MW). Nitrate–nitrite levels were then measured in the supernatant. A chemiluminescent technique for detecting nitric oxide production after reduction of nitrate and nitrite was developed to determine nitric oxide levels using a dedicated nitric oxide analyser (NOA 280i; Sievers). Protein from the above samples was analysed by Western blotting for eNOS and iNOS expression and quantified by optical densitometry.

Results: Nitrate–nitrite levels were reduced following reperfusion (mean(s.e.m.) 14·1(2·5) *versus* 9·7(1·5) µmol l^{-1}; $P = 0{\cdot}02$). Western blot analysis showed reduced levels of eNOS following reperfusion (before 0·58 *versus* after 0·2 µmol l^{-1}), although this was not statistically significant. iNOS was not detected in biopsies taken either before or after reperfusion.

Conclusion: This study demonstrates that there is a reduction in nitric oxide following reperfusion in human LT. This decrease may be related to the reduced expression of eNOS and may be of relevance in the aetiology of the tissue injury that occurs during human LT.

Prediction of outcome in hepatic resection for metastatic colorectal carcinoma

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Background: Efficacy of liver resection for metastatic colorectal carcinoma is well established based on population statistics but the outcome in an individual patient is far from certain. The aim of this study was to evaluate the ability of an artificial neural network (ANN) in predicting outcome from colorectal liver metastasis.

Methods: From January 1990 to December 1999, consecutive patients who underwent hepatic resection for colorectal metastasis were included in the analysis. Clinical and pathological prognostic variables were collected. Postoperative survival was assessed at 1, 2 and 5 years. A multilayered perceptron ANN was constructed and trained using the above data. Some 25 per cent of data sets were excluded from training and were used to evaluate the accuracy of predicting survival in individual patients using the trained ANN. The accuracy of prediction was expressed as mean squared error (MSE) and was compared with a multivariate logistic regression model.

Results: A total of 118 patients underwent hepatic resection for colorectal metastasis, of whom 86 had metachronous colorectal metastases. In the remaining 32 the lesion was synchronous to the primary colorectal lesion. The survival rate at 1, 2 and 5 years was 86, 58 and 36 per cent respectively. The perioperative mortality rate was 8 per cent. MSE of the ANN at predicting 5-year survival was 0·02, which was significantly better than the logistic regression model (MSE 0·13; $P < 0{\cdot}001$). ANN was an accurate tool at predicting 2- and 5-year survival following resection of colorectal hepatic metastasis (accuracy 92 and 96 per cent respectively).

Conclusion: ANNs are able to predict outcome following resection of colorectal metastasis in individual patients with accuracy, and outperform regression analysis. ANN is a valuable tool in the management of patients with liver metastasis from colorectal carcinoma.

Sialyl-Lewis^{x} hybridized complement receptor type 1 moderates acid aspiration injury

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Background: The potentially enhanced anti-inflammatory effects of sialyl-Lewis^{x} hybridized complement receptor type 1 (sCR1sLe^{x}) in moderating acid aspiration injury were examined.

Methods: Hydrochloric acid was instilled in tracheostomy tubes placed in anaesthetized mice and extravasation of [^{125}I]-labelled albumin in bronchoalveolar lavage (BAL) was used to calculate the vascular permeability index (PI). Polymorphonuclear neutrophil (PMN) counts in BAL and immunohistochemistry were performed. Animals were maintained in accordance with National Research Council guidelines. Statistical analysis was by *t* test with Bonferroni correction.

Results: After subtraction of the sham value, the mean (s.e.m.) PI in untreated mice ($n = 26$) was 0·09(0·01). Permeability was reduced by 44 per cent in mice treated with 5 mg kg^{-1} sCR1 ($n = 20$; PI 0·05(0·003); $P < 0·05$) and by 57 per cent when animals were treated with 10 mg kg^{-1} sCR1 ($n = 17$; PI 0·04(0·004); $P < 0·05$). Respective PI reductions in mice treated with 5 and 10 mg kg^{-1} sCR1sLe^{x} were 0·03(0·004) (67 per cent; $n = 20$) and 0·02(0·002) (82 per cent; $n = 19$), higher than values obtained for the respective sCR1-treated groups ($P < 0·05$). This result indicated PMN activity in the absence of complement activation. BAL PMN counts of 23(6) in 10 mg kg^{-1} sCR1sLe^{x}-treated mice ($n = 5$) were lower than in animals treated with 10 mg kg^{-1} sCR1 ($n = 5$; 506(148); $P < 0·05$) and in untreated mice ($n = 5$; 479(132); $P < 0·05$). This result indicated neutrophil sequestration despite inhibition of complement activation. Reductions in PI in mice treated 0·5 and 1 h after acid aspiration with 10 mg kg^{-1} sCR1sLe^{x} of 0·03(0·007) (70 per cent; $n = 19$) and 0·04(0·004) (57 per cent; $n = 20$) were greater than the decreases in PI of 0·05(0·005) (54 per cent; $n = 20$) and 0·06(0·005) (30 per cent; $n = 19$) in 10 mg kg^{-1} sCR1-treated groups ($P < 0·05$). Immunohistochemical staining for sCR1 was found only on the vascular endothelium of sCR1sLe^{x}-treated mice.

Conclusion: sCR1sLe^{x} moderates permeability by antagonizing complement activation and PMN adhesion. Delayed complement and neutrophil antagonism reduces injury.

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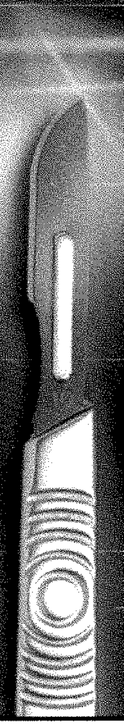

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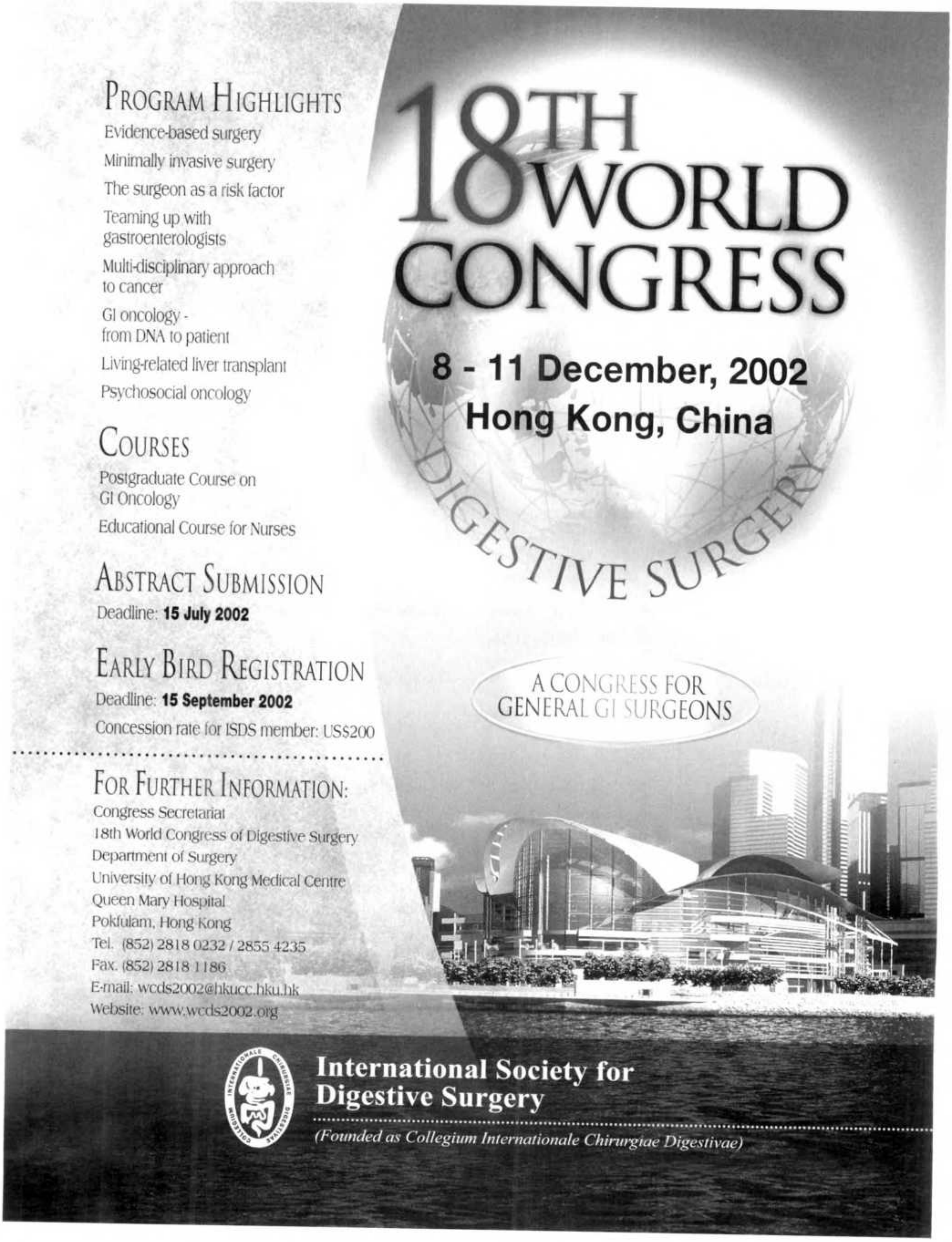

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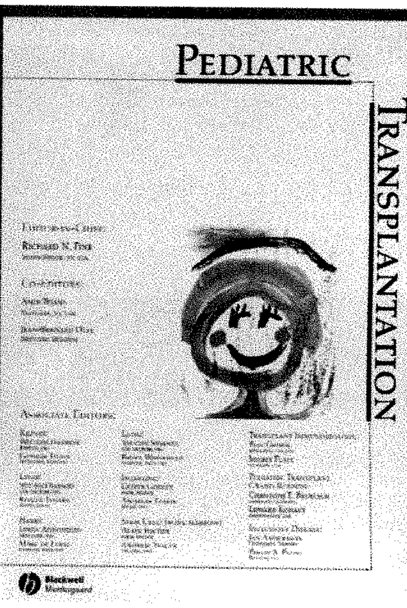

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Leading article

This article is published to coincide with the centenary celebrations of the Association of Surgeons of the Netherlands.

One hundred years of surgical science behind the dikes

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On a Sunday afternoon, 2 March 1902, at the Binnengasthuis in Amsterdam, 35 Dutch surgeons, the majority from that city, came together under the chairmanship of Professor Jacobus Rotgans (1859–1948), Professor of Surgery at the University of Amsterdam, to establish the Nederlandse Vereniging voor Heelkunde (Association of Surgeons of the Netherlands). The inaugural meeting was held at the amphitheatre of this university hospital that was, as was typical in those days, used both as a lecture hall and operation theatre for surgical demonstrations. After the official business the founding surgeons continued with the core activity of the young Association, as stated in its constitution – the enhancement of surgery and surgical science by scientific presentations, demonstrations and discussions.

The new president Rotgans was the first speaker that afternoon, presenting a paper on the upsilon-type gastroenterostomy that he had developed. Rotgans was well known for having successfully performed the first total gastrectomy for cancer in a young lady 4 years earlier. This was a formidable and dangerous novel procedure, and an example of one of the great advances in surgery, typical of the great surgeons from the German-speaking countries. Many of these surgeons came from the school of Theodor Billroth from Vienna, who had died in 1894. Dutch surgeons were very much under the influence of German surgery; they were trained in German, Austrian or Swiss institutions and German-speaking surgeons held academic positions in the Netherlands. A. Narath (1864–1924), who was a founding surgeon of the Association, was the third in a row of Billroth's trainees appointed as a professor of surgery at the University of Utrecht. Narath's needle, designed for femoral hernia repair, was used by generations of Dutch surgeons. Otto Lanz, a disciple of the famous thyroid surgeon Theodor Kocher from Berne who was awarded the Nobel prize for his work in 1908, was appointed professor of surgery in Amsterdam, where he became known for his work on appendicitis and as a connoisseur of the arts. Dutch surgeons followed their German teachers in their discipline, an authority that could be felt until the 1950s at some university departments. The Dutch used the fabulous German textbooks and high-quality detailed surgical atlases for many years.

The meetings of the Association were held four times a year (twice in Amsterdam) for about 80 years. Communications consisted in the first half of the century mainly of case reports, and demonstrations of techniques and instruments, such as the device used for gastric resection demonstrated by and named after J. Schoemaker (1871–1940) from The Hague, who was well known for his gastric resections. Discussions ensued, such as that challenging the 'red stomach' as an indication for resection, as proposed by the same Schoemaker; treatment of wounds and amputations was another frequently discussed topic, especially at the time of the Great War.

Dutch surgeons of the first half of the century very seldomly published their work in the international literature. *Nederlands Tijdschrift voor Geneeskunde* (Dutch Journal of Medicine) was the usual forum for surgical communications; for example, C. F. A. Koch (1859–1951), Professor of Surgery at the University of Groningen, produced 94 publications in this journal during his active career. In an address on the 25th anniversary of the Association, president Koch expressed his disappointment with Dutch surgery: '....by our knowledge of three languages we are able to read all publications by our neighbours....' and '....with our well-known caution we only take over those therapies that we like and thereby we prevent many of the complications encountered by others. As a result the patients are safe in our hands ....this policy is harmful for the originality of Dutch surgery. Anyway, pioneering work is seldomly encountered in our country'. This was 1927.

Two years later, Ite Boerema (1902–1980), a young trainee from the same university, visited the Mayo Clinic in the USA, an institution that had already risen to fame. He was attracted by the American spirit of the time, and by the type of surgery that was based on more original scientific ideas than on the authority of famous experts that he would meet during his further training in Germany and Holland. His trip to the USA had a great influence on his work as a teacher, scientist and professor of surgery at the University of Amsterdam from 1946 to 1973. He became known for the introduction of hyperbaric oxygen therapy, and for the development of novel techniques in the repair of hiatus hernia and in upper gastrointestinal anastomosis. He and other contemporary leaders in surgery introduced the modern surgical concepts that were developed in North America and Great Britain, and which became popular after the Second World War.

The Collegium Chirurgicum Neerlandicum was founded in 1947 to stimulate scientific surgical research. A need to have its own journal was felt and the *Archivum Chirurgicum Neerlandicum* was established in 1949 with Boerema as the first editor-in-chief. However, this publication had a marginal existence, and for many years the editors had difficulty in producing its four issues per year because of a lack of submitted manuscripts. Surgical science did not yet flourish, although various experimental laboratories were established at Dutch universities.

It was not until the 1960s and 1970s that interest in research grew among the young surgeons. This happened partly because research participation became an almost obligatory part of some of the training programmes, especially in those institutions with a research tradition of their own. The professors of the relatively new universities, such as Rotterdam (Herrie Muller 1917–1977) and later Maastricht (Jacobus Greep 1929–), sent their young trainees abroad to research fellowships in the great institutions of the USA. Many of these doctors, and a few of them later as professors of surgery, have stimulated surgical research (or better, research by surgeons) in the Netherlands. They have made Dutch surgeons aware of the impact of publications and of the attraction of scientific competition.

In 1980 the *Archivum Chirurgicum Neerlandicum* was renamed the *Netherlands Journal of Surgery* and this remained the forum of the Association of Surgeons of the Netherlands for retrospective cohort studies and interesting case reports; a few prospective studies were submitted to journals with a higher impact factor. At that time the reluctance of Dutch surgeons to embrace new therapies from other countries on the basis of retrospective studies, as was the case 50 years before, prepared an excellent seeding ground for prospective randomized clinical trials (RCTs). The technique of parietal cell vagotomy could only be implemented in Holland as therapy of choice for peptic ulcer disease when the short- and long-term outcomes of this operation had been proven to be superior over standard therapy in a RCT by Dutch and British surgeons[1,2]. The therapy became obsolete some 10 years later after original thinking by others.

In 1990 a merger between the *Netherlands Journal of Surgery* and *Acta Chirurgica Scandinavica* was proposed at the time of a number of European initiatives, such as Eurosurgery. The *European Journal of Surgery* was founded in 1992 under the editorship of Professor Sten Lennquist as the new forum of Scandinavian and Dutch surgical societies. Dutch surgeons became involved in the editorial board of the new journal, a process that has probably stimulated surgical trainees and fellows to submit their papers to that journal.

The interest in RCTs continued in many fields of surgery and when Dutch surgeons were sceptical about the concept of extended lymph node dissection, which had been shown to improve survival after gastric resection for cancer in retrospective studies, a large national randomized controlled trial was set up, in which almost all Dutch surgical groups participated under Japanese surgical guidance. The expected superiority of the D2 (extended lymph node) dissection could not be proven[3]. The effect of blood transfusion on prognosis in colorectal cancer[4], the need for antibiotic prophylaxis in surgery for fractures[5], the effect of oral anticoagulant therapy after femoropopliteal bypass surgery[6], the superiority of laparoscopic inguinal hernia repair over traditional techniques[7], and the reduction in local tumour recurrence after rectal resection with total mesorectal excision and neoadjuvant radiotherapy[8] were all studied in RCTs. They are a few examples of novel therapies, propagated by others, that have been assessed by Dutch surgeons in a prospective randomized fashion.

Lack of originality of Dutch surgeons is no longer a critical issue, as can be appreciated by the number of Dutch contributions to the biomedical literature in general and more specifically those to *BJS*. *BJS* became the forum of Dutch surgery when the membership of the Association of Surgeons of the Netherlands choose to affiliate with this Journal in 2000; the Association left the *European Journal of Surgery* at this time, albeit in good harmony. Dutch surgeons currently play a leading role within the structure of *BJS*, a publication dedicated to scientific surgery.

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Leading article

Coeliac disease

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Introduction

Coeliac disease or gluten-sensitive enteropathy has generally been considered an uncommon gastrointestinal condition; in 1950 its estimated incidence in the UK was one in 8000[1]. The advent of novel serological assays, that can be used in the initial search for coeliac disease, and endoscopic small bowel biopsy have led to increasing numbers of diagnoses[2]. Recent screening studies in several European countries now suggest that the prevalence of adult coeliac disease is of the order of one per 100–300 individuals[2]. This has been established by screening cohorts of volunteers from the general population[2].

Underdiagnosis of coeliac disease has been described by others and appears to be related to the diversity of its presentation[2]. Patients with adult coeliac disease have been thought generally to complain of gastrointestinal symptoms suggestive of malabsorption; this manner of presentation is now recognized as the classical (typical) form[2]. However, it is now known that not all patients have gastrointestinal symptoms (silent or atypical form) and that the disease may present insidiously, for example with iron deficiency anaemia, osteoporosis, cryptogenic hypertransaminasaemia, ataxia or peripheral neuropathy[2].

Despite an almost year on year increase in the number of new cases of coeliac disease, there remains a delay in its diagnosis. Studies assessing this problem have consistently described a failure to recognize the condition at both primary and secondary care levels. One recent report described a median delay in diagnosis of 4·9 (range 0·2–16) years and noted that 14 per cent of patients had had a previous gastroscopy without duodenal biopsy[3]. Those affected may have an associated morbidity with the development of coeliac-related complications, such as iron deficiency anaemia, osteoporosis, infertility, and an increased risk of gastrointestinal malignancy and certain autoimmune conditions[2]. All of these complications are potentially reversible or avoidable by adhering to a gluten-free diet.

Epidemiological surveys have suggested that irritable bowel syndrome (IBS) affects 2–22 per cent of Western populations[4]. The challenge for the clinician is to exclude organic pathology on the basis of history and clinical examination, particularly the absence of sinister features such as weight loss, rectal bleeding, nocturnal diarrhoea and anaemia. Only a small proportion of patients with putative IBS are investigated further or referred to secondary care. Gastroenterologists generally establish the diagnosis of IBS using symptom-based diagnostic criteria, particularly the Rome II Diagnostic Criteria for Functional Gastrointestinal Disorders[4]. While it is recognized that patients may have abdominal pain with altered bowel habit that does not fit the Criteria exactly, they are nevertheless used by clinicians as a diagnostic aid that represents a consensus viewpoint derived from currently available data. While not all practitioners rely on the Criteria, it has been demonstrated that they aid accurate recognition of gastrointestinal functional diagnoses in up to 80 per cent of cases[5].

A case–control study at a single university hospital assessed 300 consecutive new patients who fulfilled the ROME II Criteria for IBS, and age- and sex-matched healthy controls. The former group contained 14 patients (5 per cent) with coeliac disease, cases being recognized initially using antibody tests, with confirmation by duodenal biopsy. The control group contained two cases of coeliac disease. These findings show a significant association of coeliac disease with IBS compared with controls ($\chi^2 = 7{\cdot}6$, $P = 0{\cdot}004$, odds ratio 7·0 (95 per cent confidence interval 1·7 to 28·0))[5]. The observation that coeliac disease may be misdiagnosed as IBS should change clinical practice, and within this group of patients it seems appropriate to use antibody tests as part of the baseline laboratory profile. In addition to IBS-type symptoms, recent reports have established that coeliac disease may also present with other gastrointestinal manifestations (abdominal pain, reflux or dyspeptic symptoms) without features of malabsorption[6,7].

Pancreatic exocrine insufficiency is well described in coeliac disease but its association with idiopathic recurrent pancreatitis is a novel observation[8]. Of 169 patients referred for possible sphincter of Oddi dysfunction, 12 (7 per cent) were found to have coeliac disease with both manometric evidence of stenosis and histology consistent with periampullary inflammation and villous atrophy. The hypothetical pathophysiological mechanism is attributed to malnutrition[8].

The use of antibody profiles in the initial recognition of coeliac disease is now well established. Antigliadin antibodies (both immunoglobulin (Ig) G gliadin and A gliadin), endomysial antibody (EMA) and, more recently, tissue transglutaminase have all been evaluated. The sensitivity and specificity of these antibodies vary, but EMA appears to be the most specific with results consistently above 90 per cent[2]. However, in the above study describing the association of coeliac disease with IBS, three of the 14 cases of coeliac disease within the IBS group were EMA negative[5]. This observation is not unique. A recent study of a cohort of 69 patients with untreated coeliac disease reported the overall EMA sensitivity to be 60 per cent[9]. The Dutch group involved described a correlation between EMA positivity and the severity of the histological lesion. Patients with total villous atrophy were all EMA positive while in those with partial and subtotal villous atrophy the sensitivity of EMA testing was 31 and 70 per cent respectively. A pragmatic approach would be to use a panel of antibodies, for example both antigliadin antibodies and EMA, when investigating high-risk groups. This is economically justifiable as these tests are inexpensive (£20 for all three).

Evaluation of duodenal endoscopic markers (loss or reduction of folds, scalloped folds, mosaic or micronodular pattern) in making the diagnosis of coeliac disease, although having a specificity of over 90 per cent, has consistently been associated with a sensitivity of less than 50 per cent[10].

A case-finding approach to coeliac disease can be recommended. Patients at high risk (for example those with IBS symptoms or iron deficiency anaemia) should be investigated initially using a panel of antibodies. Histological confirmation should be obtained by quadrantic duodenal biopsy, avoiding diagnostic delay and its ensuing complications. This is a considered approach: if you look for coeliac disease you will find it.

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Consensus statement

Scientific data from clinical trials: investigators' responsibilities and rights

Based on recent reports[1–9], there are increasing concerns about the control of the scientific data obtained from clinical trials sponsored by industry. Many of the problems encountered are the result of restrictions contained in the research contracts that participating investigators are asked to sign. A number of solutions have been suggested to ensure the integrity of clinical trials, including the establishment of appropriately constituted trial oversight committees, negotiating non-interference pledges from industry sponsors, and creating proactive support of investigators' rights by organized medicine[10].

As surgical journal editors, we stand opposed to inappropriately restrictive contractual agreements governing company-sponsored clinical trials of devices or drugs, such as those containing clauses that deny the investigators proper control over the scientific aspects of the trial or restrict access to the data and their timely publication. We believe that responsibility for the scientific data from clinical trials and its analysis, interpretation, and publication should rest in the hands of the investigators. In multicentre trials, a duly appointed and properly constituted publications committee can, and usually should, carry out these responsibilities.

Recently, the editors of 13 medical journals also published their opposition to inappropriately restrictive research contracts, insisting that investigators be given and assume adequate responsibility for the conduct of a clinical trial, have sufficient access to the data to perform the necessary analyses, and have control over the decision to publish. The editors further stated that they will 'routinely require authors to disclose details of their own and the sponsor's role in the study' and '...will ask the responsible author to sign a statement indicating that he or she accepts full responsibility for the conduct of the trial, had access to the data, and controlled the decision to publish' and '...will not review or publish articles based on studies that are conducted under conditions that allow the sponsor to have sole control of the data or to withhold publication'[11].

Editors may choose not to publish an article if the sponsor had control over the trial design, data analysis, and/or publication. These requirements for publication ethics were adopted as policy on 11 May 2001, and will be included in the next publication of the *Uniform Requirements for Manuscripts Submitted to Biomedical Journals* – a document to which we are all signatories. The revised section on 'Potential Conflicts of Interest Related to Project Support' is quoted here.

> *'Increasingly, biomedical studies receive funding from commercial firms, private foundations, and government. The conditions of this funding have the potential to bias and otherwise discredit the research.*
>
> *Scientists have an ethical obligation to submit creditable research results for publication. As the persons directly responsible for their work, researchers therefore should not enter into agreements that interfere with their access to the data or their ability to analyse the data independently, to prepare manuscripts, and to publish them. Authors should describe the role of the study sponsor(s), if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the report for publication. If the supporting source had no such involvement, the authors should so state. Biases potentially introduced when sponsors are directly involved in research are analogous to methodological biases of other sorts; some journals therefore choose to include information about the sponsor's involvement in the methods section of the published paper.*
>
> *If a study is funded by an agency with a proprietary or financial interest in the outcome, editors may ask authors to sign a statement such as, 'I had full access to all of the data in this study and I take complete responsibility for the integrity of the data and the accuracy of the data analysis'. Editors should be encouraged to review copies of the protocol and/or contracts associated with project-specific studies before accepting such studies for publication. Editors may choose not to consider an article if a sponsor has asserted control over the authors' right to publish.'*

We, the undersigned surgical journal editors, support these revised guidelines and, as appropriate, will request authors of reports on clinical or basic research trials of devices and drugs to disclose details of the relative roles of the investigators and the sponsors in the conduct of the trial, the data collection and analysis, and preparation of the submitted manuscript. We may also ask the responsible author to sign a statement that he or she accepts full responsibility for the conduct of the trial, the validity of the data and its analysis, and the writing of the submitted manuscript.

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Review

Matrix metalloproteinases and atherosclerotic plaque instability

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Background: There is growing interest in the role of matrix metalloproteinases in atherosclerosis. Excessive tissue remodelling and increased matrix metalloproteinase activity have been demonstrated during atherosclerotic plaque disruption, a frequent predeterminant of ischaemic cardiac events and stroke. These enzymes represent a potential target for therapeutic intervention to modify vascular pathology.
Methods: The core of this review is derived from a Medline database literature search.
Results: There is convincing evidence of increased matrix metalloproteinase activity during acute plaque disruption. Evidence for an imbalance promoting increased matrix degradation is less well documented. However, studies of matrix metalloproteinase inhibition in models of vascular disease suggest a potential therapeutic benefit.
Conclusion: *In vivo* studies of matrix metalloproteinase inhibition are required to study the potential for reversal or deceleration of the excessive tissue remodelling that accompanies acute plaque disruption.

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Introduction

Atherosclerosis continues to cause considerable morbidity and mortality, particularly in the western world. While risk factors have been clearly identified, their precise roles in early atherogenesis are complex. Early plaque development is dependent on interactions between damaged endothelial cells, smooth muscle cells and circulating inflammatory cells by the release of cytokines, growth factors and cell adhesion molecules. Atherogenesis may represent a cell-mediated immune phenomenon, although shear stress and flow changes also have a role. Excessive connective tissue remodelling may lead to plaque instability and acute disruption of the plaque surface exposes the thrombogenic core to luminal blood flow.

Connective tissue integrity depends on a balance between degradation and repair of the extracellular matrix. The activity of proteolytic enzymes is the rate-limiting step in extracellular matrix degradation[1]. It is now well established that, among numerous other proteinases, the matrix metalloproteinases (MMPs) play the major role in the degradation of collagen and other extracellular matrix macromolecules. Expressed at low levels in normal adult tissue, MMPs are upregulated in normal and pathological remodelling processes. MMPs are intimately involved in processes such as wound healing[2], but there is growing interest in a role in disease states in which extracellular matrix breakdown is a dominant feature. Early interest focused on a pathological role for MMPs in the resorption of periodontal structures in periodontal disease[3], the destruction of joints in rheumatoid arthritis[4] and the local invasive behaviour of malignancies[5]. This has led to the development of specific inhibitors, some of which are currently being assessed in clinical trials[6].

The recognition that the balance of MMPs and their inhibitors controls the extracellular matrix has led to considerable interest in the pathological effect of MMPs in vascular disease states, in which tissue remodelling plays an important role. The atherosclerotic plaque is a dynamic structure, undergoing continuous remodelling of the extracellular matrix upon which its structural integrity depends. Acute changes within the plaque, such as intraplaque haemorrhage, cap rupture and cap ulceration, are often preludes to the onset of clinically important ischaemic events (*Fig. 1*)[7]. Each phase of the atherosclerotic process is mediated, at least in part, by MMPs[8].

The matrix metalloproteinase family

The MMPs are members of a family of proteinases characterized by tight binding of zinc ions to their active sites. There are more than 20 members of the family which, while exhibiting structural homology, are each the product of a different gene. All members of the MMP family degrade one or more components of the extracellular matrix. They are secreted as latent proenzyme forms that require activation for proteolysis.

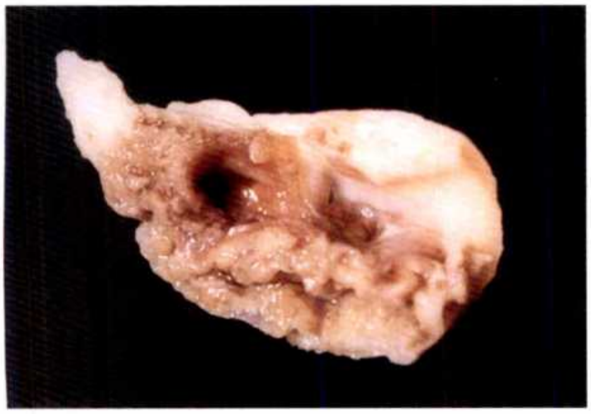

Fig. 1 Carotid plaque retrieved at carotid endarterectomy from a symptomatic patient, showing acute disruption of its surface, exposing the highly thrombogenic core

The MMPs have been divided into four major classes based broadly on substrate specificity: collagenases (MMP-1, MMP-8, MMP-13 and MMP-18), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10, MMP-11, MMP-19 and MMP-20) and membrane-type (MT) MMPs (MMP-14, MMP-15, MMP-16 and MMP-17) (*Table 1*). Many are now recognized to have broad and overlapping substrate specificities.

Each member of the MMP family has a five-domain structure, with 70 per cent similarity between members of the same group and 50 per cent similarity between members of different groups (*Fig. 2*)[9]. The first (signal) domain is essential for secretion of the peptide from the cell. The propeptide domain incorporates the 'cysteine switch' mechanism, essential for maintenance of the zymogen form[10]. The catalytic domain forms the catalytic zinc-binding site[11]. The hinge region is a small proline-rich domain thought to allow folding of the *C*-terminal domain on to the catalytic domain, while the function of the final domain is unclear.

MMP genes also show a highly conserved modular structure. All collagenase and stromelysin genes are located in a gene cluster on the long arm of chromosome 11, with the exception of MMP-11, which has been mapped to chromosome 22[12]. The MMP-2 and MMP-9 genes reside, respectively, on chromosomes 16 and 20, whereas the MT-

Table 1 Major matrix metalloproteinase subtypes and their substrates

	Enzyme	Principal known substrates
Collagenases		
MMP-1	Interstitial collagenase	Collagens I, II, III, VII, VIII and X, gelatin, aggrecan, MMP-2, MMP-9
MMP-8	Neutrophil collagenase	Collagens I, II, III, V, VII, VIII and X, gelatin, aggrecan
MMP-13	Collagenase-3	Collagens I, II, III and IV, gelatin, aggrecan, PAI-2
MMP-18	Xenopus collagenase	
Gelatinases		
MMP-2	Gelatinase A (72-kDa gelatinase)	Gelatin, collagen types I, IV, V, VII, X, XI and XIV, elastin, fibronectin, aggrecan
MMP-9	Gelatinase B (92-kDa gelatinase)	Gelatin, collagen types IV, V, VII and X, elastin
Stromelysins		
MMP-3	Stromelysin-1	Collagens III, IV, IX and X, gelatin, aggrecan, MMP-1, MMP-7, MMP-8, MMP-9 and MMP-13
MMP-10	Stromelysin-2	Collagens III, IV and V, gelatin, casein, MMP-1, MMP-8
MMP-11	Stromelysin-3	
Membrane type		
MMP-14	MT1-MMP	Collagens I,II and III, gelatin, MMP-2 and MMP-13
MMP-15	MT2-MMP	MMP-2, gelatin
MMP-16	MT3-MMP	MMP-2
MMP-17	MT4-MMP	
Others		
MMP-7	Matrilysin	Collagens IV and X, gelatin, fibronectin
MMP-12	Macrophage metalloelastase	Collagen IV, gelatin, elastin, fibronectin

MMP, matrix metalloproteinase; PAI, plasminogen activator inhibitor; MT, membrane type

MMP-1 gene is situated on chromosome 14[13]. Comparison of the MMP genes has revealed a high degree of sequence homology.

MMPs are expressed in normal tissues, especially MMP-2, although MMP-2 expression is limited to stromal cells[14]. MMP-9 is actively expressed by haematopoietic cells[15] while MMP-7 is found in the glandular epithelial cells of the gastrointestinal tract and endometrium[16]. MMP-7 is also expressed by neoplastic cells[17], along with MT-MMPs on the tumour cell membrane[18]. Most work on tumour tissue has shown that stromal cells rather than tumour cells predominantly express gelatinases and stromelysins[19].

Control of matrix metalloproteinase activity

MMP activity is tightly regulated at both intracellular and extracellular levels. Growth factors, cytokines, hormones and tumour promoters regulate MMP expression at the transcriptional level. Interleukin (IL) 1, platelet-derived growth factor and tumour necrosis factor (TNF) α have all been shown to have a stimulatory effect, while heparin, transforming growth factor (TGF) β and corticosteroids have an inhibitory effect. However, although different cell types possess the same promoters, considerable variation exists in the response to cytokine stimulation.

Extracellular activation of the latent proenzymes represents the second level of control. This is a crucial step in terms of their biological behaviour in disease states, as activation may tip the balance in favour of matrix degradation. The major physiological activator of MMPs is plasmin. The binding and activation of urokinase plasminogen activator (uPA) on the cell surface receptor is thought to localize proteolytic activity to the leading edge of the cell[20]. uPA is expressed by a variety of cells intimately involved in processes of tissue remodelling, in particular monocytes and macrophages, and inhibition of uPA *in vitro* prevents matrix degradation[21]. Smooth muscle cells and endothelial cells are also recognized to express uPA.

The subsequent stages of activation form a positive feedback loop, whereby plasmin activates MMP-1, MMP-3 and MMP-9 directly, active MMP-3 further activates MMP-1, and active MMP-1 cleaves pro-MMP-9[8]. Plasminogen activator inhibitor has an inhibitory effect on uPA, downregulating the activation cascade, and the balance between the two may be important in determining the overall level of proteolytic activity (*Fig. 3*)[22].

MMP-2 has proven resistant to activation *in vitro* by extracellular proteases, but cell membrane fractions of tumour cells were found to activate pro-MMP-2 by a mechanism sensitive to tissue inhibitors of matrix metalloproteinases (TIMPs)[23]. The major physiological activator of MMP-2 is now recognized as a member of the MT-MMP family, integral cell membrane receptors of which four types have been cloned to date[18]. Both MMP-2 and MMP-9 activity is modulated by reactive oxygen species produced by macrophage-derived foam cells. There is an increase in the steady-state levels of reactive oxygen species in a number of disease states including atherosclerosis[24], and this may directly increase MMP activation in plaques.

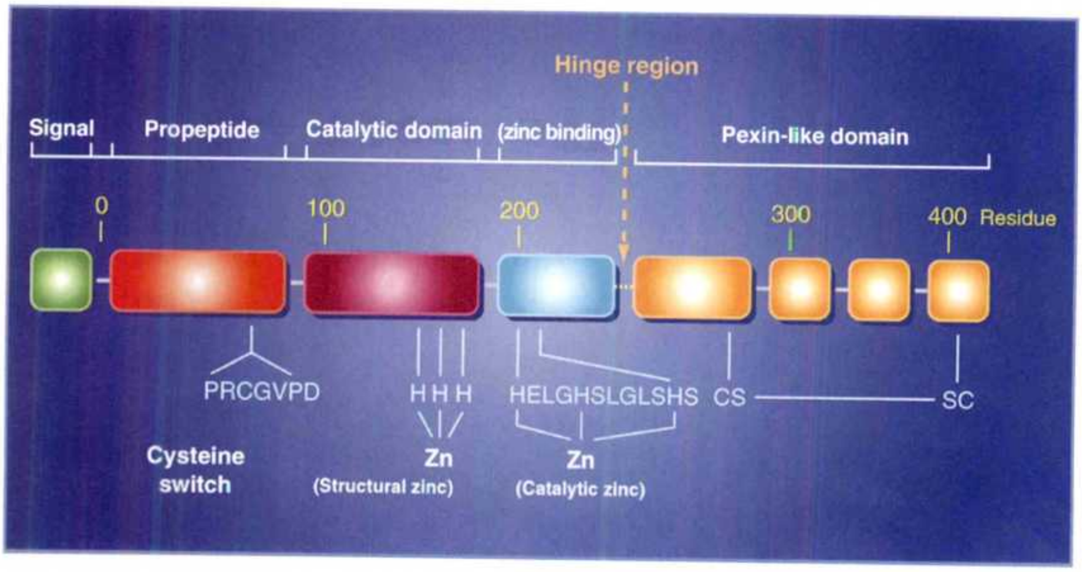

Fig. 2 Five-domain structure of the matrix metalloproteinase protein

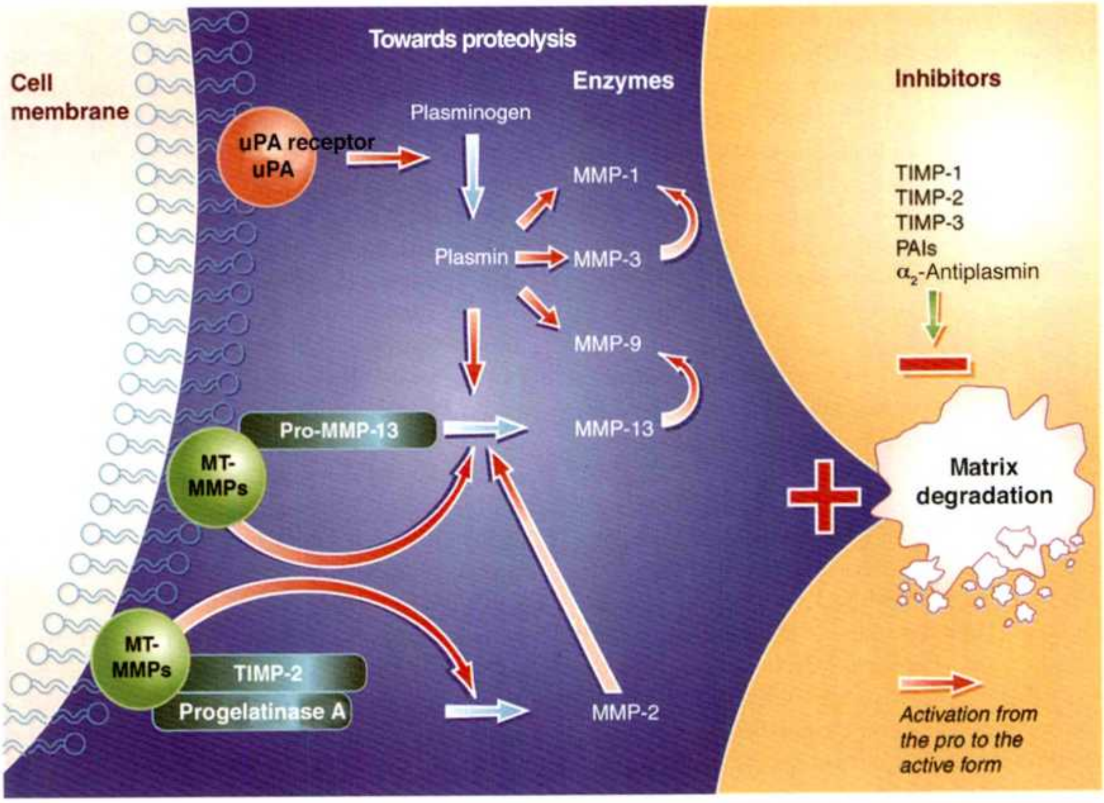

Fig. 3 Proposed interaction of various matrix metalloproteinases (MMPs) that results in a cascade of activation and matrix degradation, tightly controlled by tissue inhibitors. MT, membrane type; TIMP, tissue inhibitor of MMP; uPA, urokinase plasminogen activator

Action of tissue inhibitors of matrix metalloproteinases

A further level of control of MMP activity involves the binding of active MMPs to TIMPs. At least four in number, although structurally similar, the TIMPs share only 37–42 per cent amino acid identity, but are to an extent interchangeable in their ability to inhibit MMPs. The TIMPs inhibit MMPs by binding irreversibly to the active form of the enzyme in a 1 : 1 molar ratio[25]. A more complex interaction exists between MMP-2 and TIMP-2, and TIMP-1 and MMP-9[25,26]. Overall proteolytic activity depends on the relative concentrations of the active enzymes and their inhibitors.

Role of matrix metalloproteinases in atherosclerosis

MMPs may be involved in the early stages of plaque development. Monocyte adherence to the vessel wall is one of the earliest events in atherogenesis. On reaching the subintimal space, complex cell–cell interactions occur with the release of cytokines and growth factors. Plaque formation occurs as the result of cellular migration and proliferation accompanied by an accumulation of extracellular matrix. MMP-2-dependent basement membrane degradation promotes vascular smooth muscle cell migration in early plaque development. In a study by Pauly *et al.*[27], isolated rat vascular smooth muscle cells cultured on a gel of reconstituted basement membrane proteins secreted MMP-2, which was activated to degrade the proteins. Antiserum against MMP-2 prevented migration through the basement membrane. Similar results have been observed in a rabbit model, although direct evidence of an association in the human is lacking[28]. However, this appears to be the first limiting step in atherogenesis controlled, at least in part, by MMPs.

Acute plaque disruption

Perhaps more importantly, there is increasing evidence to suggest that MMPs are intricately involved in the destruction of connective tissue before surface disruption and thromboembolism. Acute changes within the plaque, such as intraplaque haemorrhage, cap rupture and cap ulceration, are preludes to the onset of clinically important ischaemic events[7]. Previous studies have shown that the site of plaque rupture is characterized by an intense inflammatory infiltrate[29], consisting predominantly of macrophages, foam cells and T lymphocytes. It has been hypothesized that this inflammatory infiltration plays a key role in the destabilization of the plaque[30]. The infiltrate within the plaque undergoes a period of activation at the time of acute coronary syndromes, and the associated release of proteolytic enzymes may lead to destabilization of the plaque[31]. However, the events leading up to this activation remain unclear.

There is a clear association between oxidized low-density lipoprotein (LDL) levels and atherosclerosis, possibly through a proinflammatory action, as shown by a correlation between levels and severity of acute coronary syndromes and plaque macrophages[32]. Systemic changes in inflammatory markers have been reported including white blood cell count and C-reactive protein (CRP)[33,34]. Furthermore, CRP may be involved in the uptake of LDLs by macrophages[35]. The serum levels of anti-inflammatory cytokine IL-10, a cytokine that may be involved in the regulation of MMP activity in ischaemic tissue[36], have been shown to be lower in patients with unstable angina[37]. The role of infectious agents, in particular *Chlamydia pneumoniae*, is the subject of considerable debate and requires clarification[38]. Other factors, including tissue factor and complement, may also play a part in plaque instability[39,40].

Role of matrix metalloproteinases

Early work by Vine and Powell[41] demonstrated an increase in gelatinase activity in occlusive aortic disease compared with control tissue. Zymography suggested that this was predominantly due to MMP-9. They also demonstrated collagenase and stromelysin activity in similar tissue, although this was not quantified. Henney *et al.*[42] demonstrated the presence of MMP-3 messenger RNA (mRNA) in human coronary atheroma by *in situ* hybridization, localized to macrophages in the shoulder of the plaque.

Subsequently Galis *et al.*[43] studied the expression of a variety of MMPs in human atherosclerotic plaques, and used *in situ* zymography to assess matrix-degrading activity. In non-diseased arterial tissue, smooth muscle cells expressed MMP-2 as well as TIMP-1 and TIMP-2. In contrast, atherosclerotic lesions showed immunoreactivity for MMP-1, MMP-2, MMP-3 and MMP-9 as well as the TIMPs; MMP-1, MMP-3 and MMP-9 were localized to macrophages, lymphocytes, smooth muscle cells and endothelial cells within the fibrous cap and shoulder of the plaque. *In situ* zymography demonstrated gelatinolytic and caseinolytic activity within the same areas, suggesting a potential role for MMPs in plaque matrix degradation. The levels and expression of the enzymes were not quantified in this study. Further work on MMP-1 in carotid plaques retrieved at endarterectomy demonstrated that expression was predominantly by macrophages in the cap and shoulder regions[44]. A strong correlation was shown between the percentage of lipid core staining for MMP-1 and the percentage of core occupied by haemorrhage. This represented the first attempt to relate MMP production with features of plaque instability.

Brown and co-workers[45] investigated the role of MMP-9 in acute coronary ischaemia. They performed immunostaining for MMP-9 on atherectomy specimens retrieved from 12 patients with unstable angina and compared them with those from a similar cohort with stable angina. Positive staining for MMP-9 was demonstrated in 85 per cent of both groups. However, intracellular staining, predominantly in macrophages, was seen in all of those in the unstable angina group compared with three of ten in the stable group. Macrophages do not store MMP-9[46], so this provided evidence of active enzyme synthesis in unstable plaque. The study involved small numbers and failed to provide detailed histological evidence of instability, MMP activity or TIMP levels. However, the results were supported by McMillan and co-workers[47], who demonstrated higher mRNA levels for MMP-9 in atherosclerotic aortic tissue compared with controls. Thompson and colleagues[48] provided some of the first quantitative data regarding MMP-9 in aortic tissue, using enzyme-linked immunosorbent assay (ELISA) to demonstrate a sixfold increase in MMP-9 levels in occlusive disease. TIMP-1 levels were similar to those in controls, suggesting an imbalance between enzyme and inhibitor levels.

There has been similar interest in the involvement of MMP-2 in more mature atherosclerotic lesions. Li and colleagues[49] described an increase in the level and expression of MMP-2 in human atherosclerotic aortic tissue compared with non-diseased tissue. They demonstrated increased MMP-2 activity and co-localized this to areas of macrophage infiltration. This study was limited by the sample size of only 12 individuals, collected from autopsies of young trauma victims with no history of cardiovascular disease. In a further study, Davis *et al.*[50] demonstrated significantly higher levels of MMP-9 in atherosclerotic tissue compared with controls, but MMP-2 levels lower than those in aneurysmal tissue.

More recent work has clearly demonstrated an increase in the level and expression of MMP-9 in unstable carotid plaques based on symptomatology, spontaneous embolization and histological evidence of instability[51]. In a consecutive series of 75 patients undergoing carotid endarterectomy, the plaque level of MMP-9 was found to be four times higher in patients with recent symptoms.

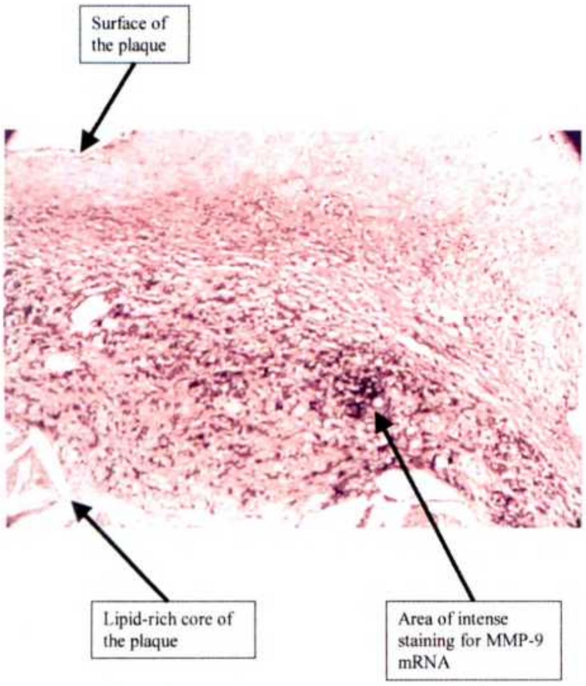
Surface of the plaque
Lipid-rich core of the plaque
Area of intense staining for MMP-9 mRNA

Fig. 4 *In situ* hybridization of matrix metalloproteinase (MMP) 9 messenger RNA (mRNA) in a plaque from a symptomatic patient, demonstrating intense expression of MMP-9 (predominantly by macrophages) in its shoulder and cap (original magnification × 50)

There was a significant increase in MMP-9 levels in plaques shown to be spontaneously embolizing before operation and plaques with histological features of acute disruption. Expression was predominantly by macrophages in the vulnerable regions of the plaque (*Fig. 4*). There were no differences in TIMP-1 levels and a significant increase in the ratio of MMP-9 to TIMP-1, suggesting an imbalance between proteolysis and inhibition.

Since the discovery of MT1-MMP and its role in the activation of MMP-2, the expression and activity of MT1-MMP has been studied in atherosclerotic plaques[52]. In normal vessels MT1-MMP expression was detected in medial smooth muscle cells and to a lesser extent in the adventitia. In atheroma MT1-MMP expression was found to co-localize with macrophages and smooth muscle cells. Cultured smooth muscle cells and macrophages were exposed to proinflammatory cytokines IL-1α and TNF-α plus oxidized LDL. TNF-α stimulated a 3·8-fold increase in macrophage expression of MT1-MMP within 6 h, while IL-1α, TNF-α and oxidized LDL exposure produced a twofold to fourfold increase in expression and protein level by cultured smooth muscle cells. This suggested that cytokines may upregulate plaque macrophage and smooth muscle cell MT1-MMP expression, influencing the activation of MMP-2 and matrix remodelling. However, no evidence was provided of a quantitative increase in MMP-2 level or activity and, while the response to cytokines *in vitro* was clear, the situation within the plaque *in vivo* is probably more complex.

Halpert *et al.*[53] have demonstrated expression of MMP-7 and MMP-12 in carotid plaques retrieved at endarterectomy. Other members of the MMP family identified within atherosclerotic tissue include MMP-3[54,55], MMP-8[56,57], MMP-12[58,59] and MMP-13[60]. The individual contribution of these enzymes towards plaque destabilization requires further clarification. Pregnancy-associated plasma protein A, another metalloproteinase that may be proatherogenic, has recently been demonstrated in unstable coronary plaques[61].

Neovascularization within the plaque may play a role in promoting destabilization[62]. The growth of capillaries during angiogenesis is influenced by MMP activity. Interactions between integrins and MMPs may control angiogenesis[63]. In particular, α(V)/β3 and β1 integrins, cell-surface extracellular matrix receptors, have been shown to play a key role in the activation pathway of MMP-2[64]. Furthermore, α(V)/β3 integrin stimulates smooth muscle cell MMP production[65] and their co-expression has been correlated with the progression of tumours[66].

Another group of enzymes with the potential to induce plaque disruption is the A disintegrin and metalloproteinase (ADAM) family, which comprise a metalloproteinase domain and an integrin-binding domain. One such enzyme is TNF-converting enzyme, which processes the inflammatory cytokine TNF-α and so may be integrally involved in the plaque inflammatory process[67]. More recently, the disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family of enzymes has been described, which has also been implicated in angiogenesis[68].

The mediators of communication between the various cell types involved in atherosclerosis are poorly understood, although there has been interest in the role of the CD40 immune mediator and its ligand CD40L. CD40L-positive T cells have been identified in human plaques and, *in vitro*, CD40/CD40L interaction induces the production of proinflammatory cytokines, adhesion molecules and proteolytic enzymes[69–71]. More recently, blocking this interaction has been shown to limit atherosclerosis *in vivo*[72] and a role in plaque instability has been suggested[73].

Evidence of an imbalance towards increased proteolysis in atherosclerosis

The mere presence and expression of MMPs within plaque does not necessarily confer an increased risk of plaque instability. However, an increase in proteolytic activity, or an imbalance between the levels of enzymes and their inhibitors, may lead to an overall increase in matrix degradation. Difficulty had arisen in investigating this concept, since the preparation of tissue extracts in which MMPs are separated from their substrate and inhibitors before assay activates the gelatinases, preventing a true assessment of the *in vivo* state[41].

Knox and co-workers[74] provided some evidence of an imbalance between MMPs and TIMPs using *in situ* zymography to identify a net increase in proteolytic activity in excess of endogenous inhibition, and comparing atherosclerotic aortic tissue with normal control aortas. This process, which is semiquantitative, involved the incubation of frozen tissue sections with fluorescently labelled substrate, areas of proteolysis appearing as dark lytic zones. On immunohistochemistry, atherosclerotic aorta demonstrated a marked increase in staining for MMP-1 and MMP-3, plus a smaller increase in TIMP-1. MMP-2 was also increased in the absence of an increase in TIMP-2. Normal tissue failed to exhibit proteolytic activity, but atherosclerotic tissue demonstrated gelatinolytic and caseinolytic activity on the luminal aspect.

Overexpression of TIMPs may well provide protection against plaque instability. In contradiction to the imbalance theory, TIMP-3 expression has been shown to be fivefold higher in plaques compared with non-diseased tissue[75]. Co-localized predominantly to macrophages, the most intense expression was identified in the vulnerable areas of the plaque, namely the cap and shoulder. More work is required to study the specific role of TIMP-3[76].

Cholesterol-fed animals developed atherosclerotic lesions that exhibited a twofold to threefold increase in TIMP-1 and TIMP-2 levels expressed by endothelial cells, smooth muscle cells and macrophages[77]. *In situ* zymography demonstrated excess MMP activity but in the areas of increased TIMP production this was less apparent. Conversely, increased TIMP-1 levels in mice have been shown to reduce plaque development[78]. Apolipoprotein E-deficient mice, which develop accelerated atherosclerosis, subjected to adenovirus-mediated overexpression of TIMP-1, demonstrated a significant decrease in the size of lesions despite identical lipid and lipoprotein profiles. There was also a significant decrease in macrophage infiltration, and MMP-2, MMP-3 and MMP-13 levels. Dietary lipid lowering has also been shown to reduce MMP-1 activity and macrophage infiltration in the aortas of rabbits[79]. Aikawa *et al.*[80] demonstrated a decrease in activity of MMP-2, MMP-3 and MMP-9 in tandem with an increase in the collagen content of the vessel wall and increased accumulation of mature smooth muscle cells.

More recently, attempts have been made to associate increased proteolysis with evidence of plaque instability. Sukhova and co-workers[81] demonstrated significantly higher levels of MMP-1 and MMP-13 in atheromatous plaques compared with fibrous lesions, and co-localization of collagenase-cleaved collagen with macrophages expressing MMP-1 and MMP-13, loss of type I collagen and increased collagenolysis. MMP-1 and MMP-13, the interstitial collagenases, are required for the initial step in collagen degradation[82]. The ratio of MMP-9 to TIMP-1 is significantly higher in carotid plaques retrieved at endarterectomy from highly symptomatic patients, plaques with histological evidence of instability and those undergoing spontaneous embolization[51].

Systemic changes in matrix metalloproteinase levels

A systemic predictor of vulnerable plaques would enable preventative intervention to be targeted at those most at risk of ischaemic events. A number of systemic factors have been investigated, in particular CRP, IL-6 and TNF-α[83]. There are a few studies on systemic changes in MMP level in atherosclerosis. In the first such study, blood levels of MMP-2 and MMP-9 were measured using ELISA in 33 patients with acute coronary syndromes, 17 with stable angina and 17 normal control subjects[84]. The level of MMP-2 in patients with stable angina was only slightly raised compared with that in controls, but patients with acute coronary syndromes had twofold higher levels. This increase was sustained for 7 days after treatment. The level of MMP-9 was increased only in those with acute coronary syndromes, twofold in myocardial infarction and threefold in unstable angina; the increase was sustained for up to 3 days and returned to control levels by day 7.

Work in patients undergoing carotid endarterectomy has detected a significant increase in circulating MMP-9 levels in those with evidence of ongoing spontaneous particulate embolization[85]. This systemic increase may relate to atherosclerotic inflammation or the degree of cerebral injury. Cerebral injury in animals is associated with increased MMP expression and recently similar evidence has been provided in human cardioembolic stroke[86]. White matter damage in vascular dementia is also associated with increased MMP activity[87]. Serial changes in blood enzyme levels may be useful in determining patients at risk. They are particularly interesting given the localized nature of plaque destabilization.

Genetic factors

It is clear that certain individuals have a susceptibility toward plaque destabilization. As discussed above, MMP expression is regulated primarily at the transcriptional level; MMP gene promoters respond differently to a variety of regulators, such as TNF-α. Sequence variations in the promoter regions may give rise to different levels of expression and so influence the degree of extracellular matrix degradation. This has led to considerable interest in the study of such genetic variations, since they may increase susceptibility to the development and complications of atherosclerosis.

Ye and colleagues[88] detected a common polymorphism in the MMP-3 promoter region in which one allele sequence had five adenosines (5A) and the other had six adenosines (6A). Work *in vitro* showed that the 5A allele had a twofold higher promoter activity than the 6A allele, and so may lead to less extracellular matrix accumulation and tip the balance in favour of degradation[89]. Subsequently, Terashima and co-workers[90] studied the link between the 5A/6A polymorphism and acute myocardial infarction by conducting a case–control study of 330 patients with myocardial infarction and 330 control subjects. They demonstrated that the prevalence of the 5A/6A and 5A/5A genotype was significantly greater in patients with a myocardial infarction than in the control population (49 and 33 per cent respectively; $P < 0.001$). This was independent of all other cardiovascular risk factors, suggesting a possible genetic susceptibility to plaque rupture. A genetic variation in the IL-6 gene promoter has been identified which, in combination with the 5A/6A polymorphism, increased the degree of intima–media thickening at the carotid bifurcation[91].

A polymorphism has been detected in the MMP-9 gene promoter region, arising from a cytosine to thymidine transition[92]. The thymidine allele has a higher promoter activity than the cytosine allele. Genotype studies of 584 men after myocardial infarction showed a significant increase in the frequency of the thymidine allele in those with angiographic coronary artery stenosis of more than 50 per cent diameter. To date, no association has been identified with features of plaque instability.

More recently, a number of genetic variations have been described in the MMP-2 gene[93]. One variant in the promoter region demonstrated a dramatically lower promoter activity. The frequency of this polymorphism in patients with vascular disease is yet to be published.

Matrix metalloproteinase inhibition

Most of the work on MMP inhibition has been in the fields of arthritis, periodontal disease and cancer[8]. In vascular disease, most studies have been performed to investigate the effect on intimal hyperplasia and aneurysm formation. However, they provide useful evidence of the effect of targeted MMP inhibition in vascular tissue. The hypothesis behind MMP inhibition is twofold; first, that cellular migration is MMP dependent, inhibition trapping the cell in its matrix surround, and, second, that an imbalance between enzymes and inhibitors leads to excessive tissue degradation as noted in unstable plaques. Inhibition may redress the balance back in favour of matrix accumulation. Potential methods of inhibition include increasing TIMP levels, administration of inhibitors of MMP activity and reducing MMP production.

Tissue inhibitors of matrix metalloproteinases

TIMP levels could be increased by the exogenous administration of recombinant TIMPs or stimulating increased expression through gene therapy. Work on TIMPs has been restricted to animal models and *in vitro* culture of human vascular cells, predominantly investigating beneficial effects in terms of reducing intimal hyperplasia[94] and aneurysm development[95]. Increased levels of TIMP-1 in mice have been shown to reduce the development of atherosclerotic lesions[78]. Also in an animal model adenovirus-mediated overexpression of TIMP-1 led to a significant decrease in the size of atheromatous lesions despite identical lipid and lipoprotein profiles[78]. More recently, local gene transfer of TIMP-2 in a mouse vein graft model decreased vascular remodelling in tandem with a decrease in MMP activity[96].

There are a number of potential problems in extrapolating these data to the human situation. Tissue delivery of TIMPs poses a major drawback, since exogenous products would be metabolized and denatured with minimal tissue penetration at the intended site of action. Treatment would have to be in the form of local tissue delivery or gene therapy.

Synthetic inhibitors

A number of synthetic inhibitors have been investigated, in particular specific synthetic peptides, tetracycline-based antibiotics and anthracyclines. Synthetic peptides are largely analogues of the cleavage site in the collagen molecule with a metal-binding group in the position of the cleaved peptide bond. The metal-binding group binds to the zinc atom at the active site of the MMP[97].

One of the first synthetic peptides was batimastat, which has a broad-spectrum inhibitory action against the MMPs via a direct toxic action. This showed promising results in prevention of tumour development and metastasis in animal models[98], and in limiting aneurysm expansion in experimental models[99]. Batimastat has been tested in clinical trials, although its long-term use is precluded by its lack of oral bioavailability[5].

Marimastat is a second-generation inhibitor with a similar structure and mode of action to batimastat. Unlike batimastat, marimastat is active orally[6]. Preliminary results in cancer were promising, although concerns were raised about significant musculoskeletal side-effects in 30 per cent of patients[100]. Marimastat has been studied in human experimental models of intimal hyperplasia and aneurysms with encouraging results[101,102].

Concern about the long-term use of such compounds in view of their relatively broad spectrum of activity and potential side-effects has led to the development of more specific inhibitors[103], including orally acting specific inhibitors of MMP-2 and MMP-9[104]. Results of their use, and other specific synthetic inhibitors, in models of vascular disease are awaited with interest.

Tetracyclines

Doxycycline is a member of the tetracycline antibiotic family that, apart from its antibacterial action, is also a potent non-selective MMP inhibitor. Its main mode of action is through binding to the active zinc site, although it also binds to a non-active calcium site resulting in conformational change in the enzyme structure with loss of activity[105]. It may also suppress MMP expression[106]. Coupled with their low cost, clinical availability and good safety profile, the tetracyclines are an attractive option in studies of MMP inhibition.

Most work in clinical trials has been in the field of periodontal disease, where doxycycline therapy has led to clinical improvement associated with a reduction in collagenase activity[107]. In patients with osteoarthritis, oral administration of doxycycline before hip surgery reduced cartilage collagenase and gelatinase activity[108]. Doxycycline treatment leads to a reduction in MMP-1 and MMP-13 production, possibly through a regulatory effect on cytokine and cytokine receptor synthesis[109]. It has also been shown to reduce malignant cell proliferation and MMP-1 activity in a canine model of osteosarcoma[110].

In vitro, doxycycline prevents intimal hyperplasia in an established human vein culture model, by inhibition of MMP activity[111]. It also prevents aneurysmal degeneration in the elastase-induced rat model of abdominal aortic aneurysm, causing elastin preservation associated with reduced MMP-9 activity[112]. Similar results have been obtained using chemically modified tetracyclines without antibiotic activity, compounds that may prove to have even fewer side-effects than doxycycline and with no potential for encouraging bacterial resistance in long-term use[113].

Intravenous tetracycline given to patients before aneurysm surgery rapidly penetrated the arterial wall[114]. More importantly, patients who received treatment with oral low-dose doxycycline for 7 days before operation exhibited a threefold reduction in the aortic wall expression of MMP-2 and a fourfold reduction in expression of MMP-9[115]. On the basis of these results the authors called for a prospective randomized controlled trial to assess the effect of long-term treatment on the expansion of small aneurysms.

The results of a current randomized clinical trial of doxycycline *versus* placebo in patients undergoing carotid endarterectomy are awaited, but large-scale randomized trials will be required to investigate the full potential benefit of doxycycline in atherosclerosis.

Other inhibitory agents

MMP expression is downregulated by TGF-β, retinoids and corticosteroids. However, *in vivo* their practical use is limited and the results obtained variable. For example, heparin reduces expression of MMP-3 and MMP-9, but neither eliminates activity nor prevents atherosclerosis[8]. The alternative approach via application of antisense oligonucleotides to the MMP genes is possible, but clinical application awaits more detailed study of the potential effects, both beneficial and harmful.

Another possible method of interrupting the MMP pathway is to target the inflammatory response. One specific target is prostaglandin E_2, which regulates the expression of MMP-9. Prostaglandin E_2 has adverse effects on smooth muscle cell viability and cytokine secretion *in vitro*, and indomethacin has been shown to reduce cytokine release and prostaglandin production in aortic explants[116]. Indomethacin, by reducing the synthesis of prostaglandin E_2, also decreased the production of MMP-9 in the rat[117]. A case–control study demonstrated a lower rate of aneurysm growth in patients taking non-steroidal anti-inflammatory drugs compared with controls, suggesting a potentially effective treatment for preventing aneurysm expansion[118]. No such studies have been performed in patients with coronary or carotid atherosclerosis. Other non-steroidal drugs are known to modulate MMP activity, but their action *in vivo* requires detailed clinical study[119].

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors

There has been considerable interest in the beneficial effect exerted by 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors in coronary and cerebrovascular disease[120]. The beneficial action of such inhibitors seems to be multifactorial, with lipid-lowering effects in addition to direct actions on vessel wall cell–cell interactions and MMP production. This class of drugs offers the exciting potential for modification of matrix regulation in vascular disease.

A prospective study in patients with a history of myocardial infarction and raised levels of LDL cholesterol treated with fluvastatin demonstrated a 32 per cent reduction in stroke risk[121]. Also, fluvastatin reduced the progression of angiographically determined coronary artery disease and improved event-free survival, compared with placebo treatment[122]. Similar encouraging results have been obtained with other members of the statin family[123,124]. High-resolution magnetic resonance imaging has demonstrated significant plaque regression after 12 months of treatment with simvastatin[125]. Initially, statins were believed to act through their lipid-lowering ability, but the situation now seems to be more complex[126]. Two members of the group, fluvastatin and simvastatin, are known to inhibit smooth muscle cell migration and proliferation, independent of their lipid-lowering activity[127]. It is likely that there exists a direct antiproliferative effect on the arterial wall[128]; fluvastatin treatment in a rat model of intimal hyperplasia prevented neointima formation in this way[129].

In vitro work has shown that HMG CoA reductase inhibitors directly interfere with the adherence of monocytes to endothelium by reducing the expression of monocyte cell surface receptors, again independent of their lipid-lowering effect[130]. This has also been shown in parallel with a downregulation in P-selectin expression on endothelial cells[131]. Moreover, both fluvastatin and simvastatin inhibit macrophage secretion of MMP-9 *in vitro*[132]. Simvastatin is also known to exert anti-inflammatory and cardioprotective effects in apolipoprotein-E-deficient mice[133].

Cerivastatin suppresses the growth of macrophages in rabbits[134], while fluvastatin and pravastatin reduce MMP-1, MMP-3 and MMP-9 expression in a similar model, with preservation of collagen[135]. Pravastatin treatment before carotid endarterectomy reduces plaque lipid content, inflammation, MMP activity and cell death, in tandem with an increase in collagen content[136]. Statin treatment has been shown in two separate studies to confer a significant, and lipid-independent, reduction in CRP level associated with the severity of atherosclerosis[137,138].

Conclusion

While many of the factors that predispose to the early development of atherosclerotic lesions have been identified, there remains an uncertainty why, after years of indolent growth, a plaque should suddenly undergo the acute changes that herald the onset of symptoms. There is mounting evidence associating increased levels and activity of MMPs within plaques and plaque instability. There are also convincing data to suggest that an imbalance between MMP levels and their inhibitors may tip the equilibrium from matrix accumulation towards matrix degradation. This localized increase has the potential to cause the acute plaque disruption that precedes the onset of both coronary and cerebral symptoms. MMPs represent an attractive target for pharmacotherapy to prevent plaque destabilization; a variety of pharmaceutical agents is known to inhibit MMP activity. Nevertheless, a true causal relationship will only be deduced from a formal randomized clinical trial of an MMP inhibitor in patients with significant carotid or coronary stenosis.

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Systematic review

Systematic review of the use of fibrin sealant to minimize perioperative allogeneic blood transfusion

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Background: Fibrin sealants have become popular in improving perioperative haemostasis and reducing the need for allogeneic red cell transfusion.

Methods: A systematic review of randomized controlled trials was conducted to examine the efficacy of fibrin sealants in reducing perioperative blood loss and allogeneic red blood cell transfusion. Studies were identified by computer searches of Medline, Embase, Current Contents, the Cochrane Library, manufacturer websites (to January 2001), and bibliographic searches of published articles. Trials were eligible for inclusion if they involved adult elective surgery and reported quantitative data on blood loss, the proportion of patients exposed to allogeneic red cell transfusion and/or the volume of blood transfused.

Results: Twelve trials met the criteria for inclusion. Fibrin sealants reduced the rate of allogeneic blood transfusion (relative risk 0·40 (95 per cent confidence interval (c.i.) 0·26 to 0·61); five trials with 275 subjects) and reduced blood loss (weighted mean difference −151·68 (95 per cent c.i. −251·91 to −51·46) ml; seven trials with 391 subjects). Generally, the trials were small and of poor methodological quality.

Conclusion: Overall the results suggest that fibrin sealants are efficacious. Owing to lack of blinding, transfusion practices may have been influenced by knowledge of the patient's treatment status. This raises concern about blood transfusion practice as a response variable. Large methodologically rigorous trials of fibrin sealants with clinical outcomes are needed.

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Introduction

Red blood cell transfusion is an extremely common medical intervention and, of all red blood cell units transfused, between 60 and 70 per cent are used in the surgical setting[1,2]. Worldwide concern about the safety of transfused blood has prompted a reconsideration of the role of allogeneic blood transfusion (whole blood or packed red cells from an unrelated donor). Nevertheless, the evidence on the benefits, harms and costs of a range of techniques designed to minimize the use of this scarce resource needs to be examined critically, as some of the alternatives to allogeneic blood have their own risks and are expensive[3,4].

Fibrin sealants have been used in surgery for over 20 years[5]. They generally contain two major components, fibrinogen (with or without factor XIII) and thrombin (plus calcium with or without antifibrinolytic drugs), which can be applied to the wound surface sequentially or simultaneously, using single- or dual-syringe systems in a liquid or aerosolized form. Fibrin sealants mimic the final phase of the coagulation cascade through the activation of fibrinogen by thrombin which, via a series of complex reactions, leads to the formation of a semirigid clot[6]. Recognition of their potential as haemostatic agents has seen their use expand across a range of surgical settings, including cardiovascular, orthopaedic, thoracic and orthodontic surgery[7].

Reviews of fibrin sealants are abundant, but the majority are narrative texts that primarily provide information on scientific or technical aspects[5–14]. To date, there has been no systematic review of fibrin sealants that uses the methodology established by the Cochrane Collaboration[15]. The purpose of the present work was to find, appraise and summarize the data from randomized controlled trials that studied the impact of fibrin sealants on perioperative blood loss and allogeneic red blood cell transfusion in adult elective surgery.

Methods

The primary objective of this study was to determine whether fibrin sealant use in elective surgery decreases the proportion of adult patients who require perioperative allogeneic red cell transfusion. Secondary analyses included the number of units transfused per patient, blood loss (assessed objectively), the frequency of reoperation owing to bleeding, the frequency of wound infection, wound dehiscence, thrombosis and death, and length of stay in hospital.

Literature search and study selection

This review was undertaken using the standard methodology for systematic reviews established by the Cochrane Collaboration[15], and represents one of a series of related reviews undertaken by the Cochrane Injuries Group[16–19]. Databases searched were: Medline (1966 to January 2001), Embase (1980 to January 2001), Current Contents (1993 to January 2001) and the Cochrane Controlled Trials Register (Issue 1, 2001). These databases were searched initially using unrestricted search strategies, employing exploded MeSH terms and specific text-word terms for fibrin sealant. The exploded MeSH terms included: 'fibrin tissue adhesive' and 'fibrin glue'. The specific text-word terms included: 'fibrin glue$', 'fibrin sealant$', 'fibrin seal$', 'biological glue$ or biological seal$.tw', 'beriplast', 'bolheal', 'tissucol', 'tisseel', 'quixil' and 'biocol'.

To restrict and improve the specificity of these searches, two search filters were used. First, a filter developed by the International Study of Perioperative Transfusion group[20] was employed that identifies blood transfusion trials using the MeSH terms: 'blood transfusion', 'hemorrhage' and 'anesthesia'. This filter combines these MeSH terms with the text-word terms 'transfusion$', 'bleed$', 'blood loss$' and 'haemorrhag$'. Second, a modified version of the Cochrane Collaboration filter[21] was used to identify randomized controlled trials. These search filters were combined with the specified MeSH terms and the relevant text-word terms for fibrin sealant. In addition to these computer database searches, manufacturer websites were searched and experts in the field were contacted to identify relevant reports or projects in progress relevant to the review. The bibliographies of eligible trials, review articles and reports were searched for potentially relevant studies.

Only randomized controlled trials that described the proportion of patients exposed to allogeneic red blood cells and/or provided quantitative data on blood loss were included. Trials that provided only subjective assessment of blood loss (e.g. opinion expressed by the surgeon or anaesthetist) were not considered for review. Studies were included if they were published in English, German, French or Italian. Duplicate publications, studies involving children (less than 18 years of age) and studies involving non-elective surgery were excluded. Only those studies that investigated the use of fibrin sealant delivered directly to the wound surface in either liquid or aerosolized form were considered. Trials that assessed bandages or pads impregnated with lyophilized fibrin sealant components were not considered.

Data collection

The primary outcome measures were the proportion of patients exposed to allogeneic blood transfusion, volume of blood transfused (expressed as units of blood) and blood loss (expressed in millilitres). Other outcome measures were frequency of reoperation for bleeding, any infection, wound infection, wound dehiscence, thrombosis, death and length of hospital stay. Data were stratified by the type of surgery, the type of fibrin sealant used and the presence of transfusion protocols.

Studies that met the inclusion criteria were assessed for methodological quality by two independent raters. Disagreements were resolved by consensus. The methodological quality was assessed using criteria proposed by Schulz *et al.*[22]. The Schulz criteria contain questions about four items: double blinding, allocation concealment, participant inclusion/exclusion and methods used to achieve randomization.

Data analysis

Dichotomous data (proportions of patients transfused, with infection, with thrombosis, requiring reoperation for bleeding) and continuous data (mean units of blood transfused and mean volume of blood loss) were analysed using Review Manager 4.1 (MetaView 4.1)[23]. If standard deviations or standard errors of means were not reported for continuous data the study was not included in the analysis. Data expressed in ml for the volume of blood transfused were converted to units by dividing by 300. Outcomes were expressed as pooled relative risks (RRs), risk differences or weighted mean differences (WMDs) (for continuous variables) using a random effects model. In the presence of heterogeneity the random effects model provides a more conservative interpretation of the effect size, as the calculated confidence intervals tend to be wider[24]. The presence of heterogeneity of treatment effect was assessed using the Q statistic, which has an approximate χ^2 distribution with degrees of freedom equal to the number of studies minus 1[24]. $P \leq 0.1$ was used to define statistically significant heterogeneity.

Results

The literature search identified 14 trials of fibrin sealant in adult elective surgery that reported information on the main outcomes of interest. Of these, 12 fulfilled the inclusion criteria. The two trials that were excluded[25, 26] used actively treated (fibrin sealant) control groups.

Characteristics of included studies

The 12 trials included in the review[27–38] recruited a total of 624 patients of whom 291 were randomized to receive fibrin sealant (*Table 1*). The trials were small, with fewer than 50 patients in each arm. The trials were conducted in Germany ($n = 2$), Denmark ($n = 2$), Japan ($n = 2$), France ($n = 2$), Taiwan (Republic of China) ($n = 1$), UK ($n = 1$), USA ($n = 1$) and Israel ($n = 1$). Nine trials were published in English, two[34,38] in German and one in French[36]. The non-English studies were translated before being included in the analysis.

The trials were heterogeneous in terms of surgical setting and type of fibrin sealant investigated (*Table 1*). The sealants varied markedly in composition. The fibrinogen content ranged from 29 to 115 mg/ml and the thrombin concentration ranged from 200 to 1000 units/ml. The use of antifibrinolytic agents also varied with the different agents studied. The aprotinin content ranged from 1000 to 10 000 kallikrein inhibitor units per ml. The fibrin sealant Quixil™ (Omrix Biopharmaceuticals SA, Nes-Ziona, Israel) contained tranexamic acid 95 mg/ml as the antifibrinolytic agent. Where studies failed to provide product details, manufacturer websites were used to source information pertaining to fibrin sealant components.

Methodological quality of studies

Nine of the 12 trials were assessed for methodological quality by two raters. As the three non-English studies[34,36,38] could not be assessed adequately by two raters, they were excluded from the analysis of the reliability of quality assessment procedure. The inter-rater agreement for the assessment of methodological quality was generally good. For the four items of the Schulz criteria used to assess trial quality, the observed agreement was statistically significantly better than chance. Kappa scores ranged from 0·61 to 1·0, being lowest in the case of the items measuring blinding ($\kappa = 0{\cdot}61$) and concealment of treatment allocation ($\kappa = 0{\cdot}78$).

The methodological quality of the nine trials was poor. Full blinding of outcome assessment did not occur in any study, although partial blinding (single blinding) was reported in one trial[28]. Six of the nine trials failed to report the method used to generate allocation sequences (randomization process). Of those trials that described the concealment of treatment allocation, one reported using central randomization[29] and two reported using randomization codes placed in sealed envelopes[30,31]. The latter method has the potential to be unmasked, leading to selection bias in the inclusion of patients in trials[22]. Follow-up appears to have been complete in all but two trials[28,35], and even these reported only a small number of exclusions making differential withdrawal an unlikely source of bias. Because of the small number of trials no formal assessment of the relationship between trial quality and effect size was performed.

Meta-analyses

Exposure to allogeneic blood transfusion

Five trials of various types of fibrin sealant reported data on perioperative exposure to allogeneic blood transfusion (*Fig. 1*). These trials evaluated a total of 275 patients, of whom 142 were randomized to fibrin sealant. In patients who received fibrin sealant treatment the pooled RR of exposure to allogeneic blood transfusion was 0·40 (95 per cent confidence interval (c.i.) 0·26 to 0·61), equivalent to a 60 per cent RR reduction. The absolute risk of requiring a transfusion with allogeneic blood was reduced by 22 (95 per cent c.i. 9 to 36) per cent, equivalent to a number needed to treat of 4·5. Heterogeneity between these trials was not statistically significant ($P = 0{\cdot}54$).

Volume of blood transfused

Four trials reported data on volume of blood transfused (*Fig. 2*). These trials included a total of 244 patients, of whom 112 were randomized to fibrin sealant treatment. The WMD for the number of units transfused in patients treated with fibrin sealant compared with controls was − 0·56 (95 per cent c.i. − 0·84 to − 0·29) units. It should be noted that this calculation includes data from patients who did not receive blood (zero values were included). Heterogeneity between these trials was not statistically significant ($P = 0{\cdot}67$).

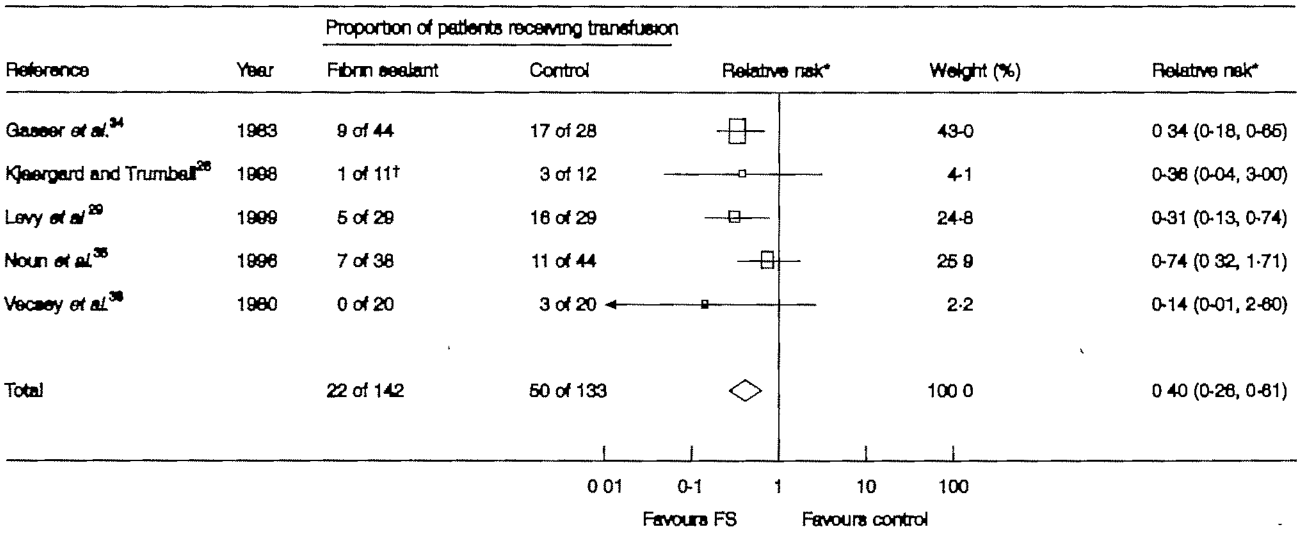
Blood loss

Seven trials, including a total of 391 patients, provided intraoperative and/or postoperative blood loss data (*Fig. 3*). In aggregate, the WMD for total blood loss was − 151·68 (95

Table 1 Randomized controlled trials of fibrin sealant

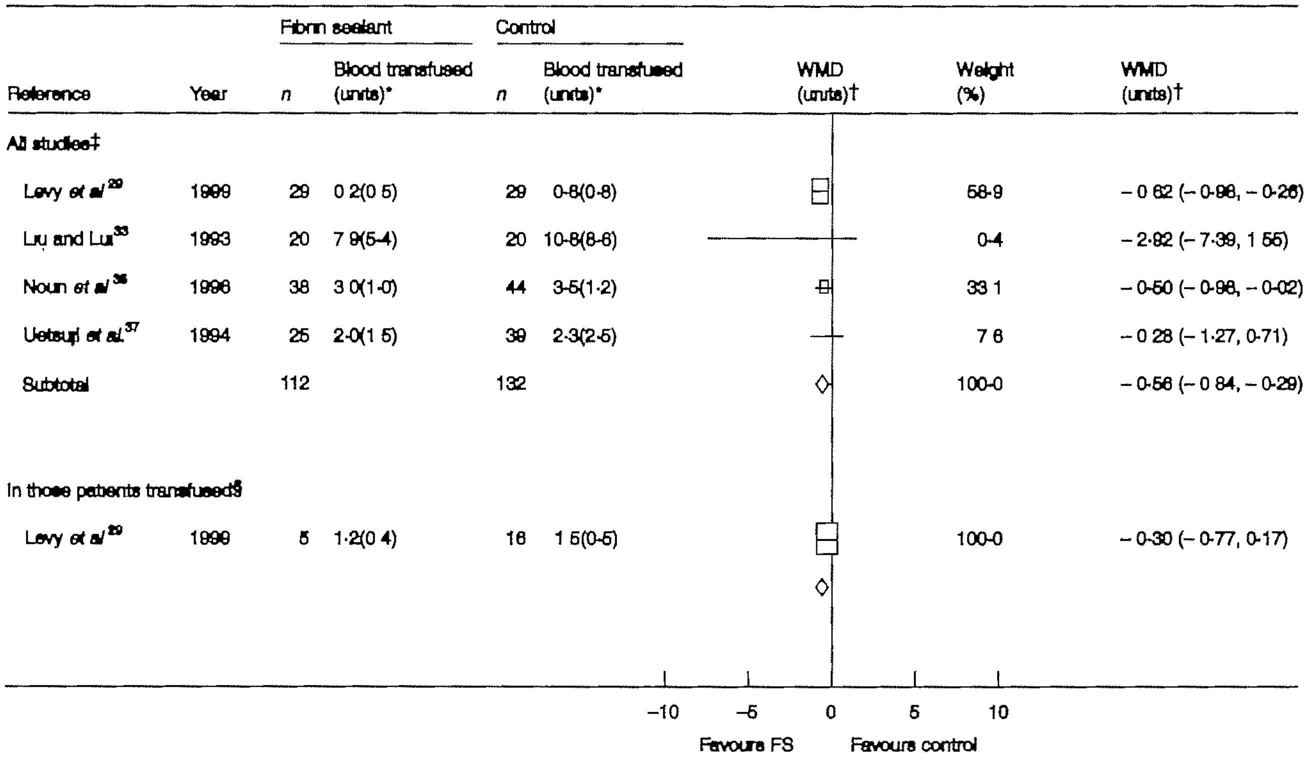
Reference	Year	Country	Source of fibrin sealant	Type of surgery	Interventions	Outcome
Vecsey[38]	1980	Germany	Fibrinogen (Immuno*) Thrombin (Hoffman-La Roche†)	Prostatectomy	FS ($n = 20$) *versus* control ($n = 20$)	24-h postoperative EBL: FS 18(21) ml *versus* control 90(108) ml ($P < 0·05$) Patients transfused: FS none *versus* control 15%
Gasser *et al.*[34]	1983	Germany	Fibrinogen (Immuno*) Factor XIII (Beringwerke‡) Thrombin (Hoffman-La Roche†)	Prostatectomy	FS + CF ($n = 44$) *versus* CF ($n = 28$) *versus* control ($n = 38$)	Total postoperative blood loss: FS + CF 60(68) ml *versus* CF 83(70) ml ($P = 0·17$) Blood transfused: FS + CF 0·25 units *versus* CF 0·72 units
Luke *et al.*[27]	1986	Denmark	Beriplast® (Beringwerke‡)	Prostatectomy	FS ($n = 15$) *versus* control ($n = 15$)	72-h postoperative blood loss: FS 46(36) ml *versus* control 249(278) ml ($P < 0·01$)
Wurtz *et al.*[36]	1991	France	Biocol® (CRTS§)	Partial pulmonary excision	FS ($n = 25$) *versus* electrocoagulation ($n = 25$)	24-h postoperative blood loss: FS 501(201) ml *versus* control 467(263) ml ($P = 0·61$)
Kohno *et al.*[31]	1992	Japan	Beriplastp® (Beringwerke‡)	Hepatic resection	FS ($n = 31$) *versus* microcrystalline powder ($n = 31$)	24-h postoperative blood loss: FS 164(147) ml *versus* control 156(177) ml ($P = 0·85$)
Liu and Lui[33]	1993	Taiwan	Beriplastp® (Beringwerke‡)	Hepatic resection	FS ($n = 20$) *versus* control ($n = 20$)	Intraoperative blood transfused: FS 7·9(5·4) units *versus* control 10·8(10·8) units ($P = 0·21$) Intraoperative EBL: FS 3047(2142) ml *versus* control 4052(3184) ml ($P = 0·25$)
Uetsuji *et al.*[37]	1994	Japan	Tissucol® (Immuno*)	Hepatectomy + liver mobilization	FS ($n = 25$) *versus* control ($n = 39$)	Blood transfused: FS 2·0(1·5) units *versus* control 2·3(2·5) units ($P = 0·62$) Intraoperative EBL: FS 1420(1101) ml *versus* control 1626(1404) ml ($P = 0·54$)
Milne *et al.*[32]	1995	UK	Fibrinogen (SNBTS¶) Thrombin (CRTS§)	Carotid endarterectomy	FS ($n = 8$) *versus* control ($n = 9$)	Median (range) blood loss: FS 420 (300–500) ml *versus* control 550 (350–1200) ml
Noun *et al.*[35]	1996	France	Biocol® (CRTS**)	Hepatic resection	FS ($n = 38$) *versus* control ($n = 44$)	Patients transfused: FS 18% *versus* control 25% Blood transfused: FS 3·0(1·0) units *versus* control 3·5(1·2) units
Kjaergard and Trumball[28]	1998	Denmark	Autologous: Vivostat™ system (Conva Tec††)	CABG	FS ($n = 12$) *versus* control ($n = 12$)	72-h postoperative blood loss: FS 242(249) ml *versus* control 505(666) ml ($P < 0·05$) Patients transfused: FS 9% *versus* control 25%
Jackson *et al.*[30]	1999	USA	Purified human FS (Baxter Healthcare‡‡)	Carotid endarterectomy	FS ($n = 24$) *versus* thrombin-soaked gelatin sponge ($n = 23$)	Median (range) blood loss: FS 720 (420–1765) ml *versus* control 733 (200–3200) ml ($P > 0·4$) Intraoperative EBL: FS 105(108) ml *versus* control 94(121) ml ($P = 0·55$)
Levy *et al.*[29]	1999	Israel	Quixil™ (Omrix Biopharmaceuticals SA§§)	Total knee arthroplasty	FS ($n = 29$) *versus* control ($n = 29$)	Patients transfused: FS 17% *versus* control 55% ($P = 0·004$) Total blood loss (drainage): FS 360(288) ml *versus* control 878(403) ml ($P < 0·001$)

Values are mean(s.d.) unless otherwise stated. FS, fibrin sealant; CF, collagen fleece; EBL, estimated blood loss; CABG, coronary artery bypass graft; SNBTS, Scottish National Blood Transfusion Service; CRTS, Central Regional de Transfusion Sanguine. *Vienna, Austria; †Germany; ‡Marburg, Germany; §Lille, France; ¶Edinburgh, UK; **Les Ulis, France; ††Princeton, New Jersey, USA; ‡‡Glendale, California, USA; §§Nes-Ziona, Israel



Reference	Year	Proportion of patients receiving transfusion: Fibrin sealant	Control	Weight (%)	Relative risk*
Gasser *et al.*[34]	1983	9 of 44	17 of 28	43·0	0·34 (0·18, 0·65)
Kjaergard and Trumbell[28]	1998	1 of 11†	3 of 12	4·1	0·36 (0·04, 3·00)
Levy *et al*[29]	1999	5 of 29	16 of 29	24·8	0·31 (0·13, 0·74)
Noun *et al.*[35]	1996	7 of 38	11 of 44	25·9	0·74 (0·32, 1·71)
Vecsey *et al.*[36]	1980	0 of 20	3 of 20	2·2	0·14 (0·01, 2·60)
Total		22 of 142	50 of 133	100·0	0·40 (0·26, 0·61)

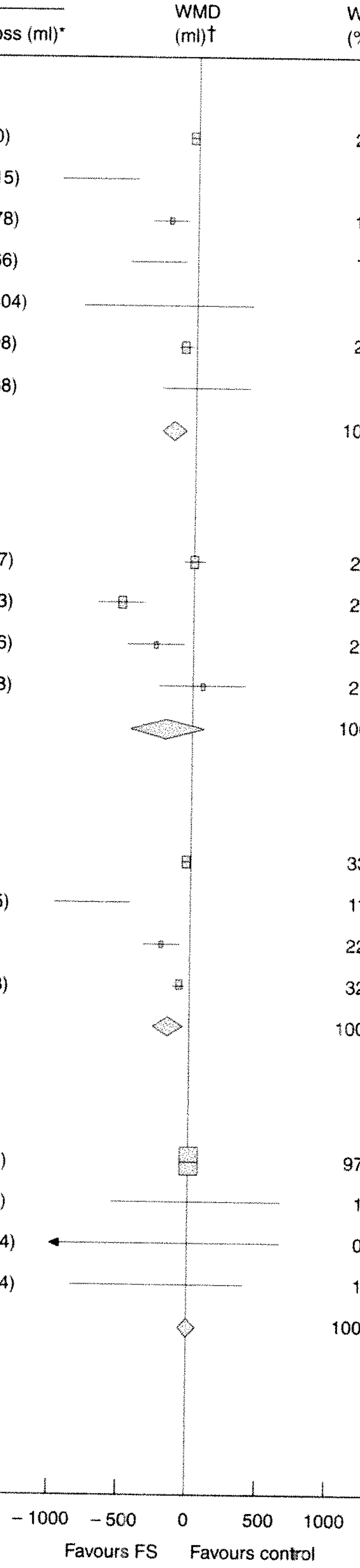
Fig. 1 Proportion of patients receiving transfusion of allogeneic red cells. *Values are relative risk with 95 per cent confidence interval. †There were 12 patients in this group, however, one patient was withdrawn from the trial. FS, fibrin sealant. Test for heterogeneity $\chi^2 = 3{\cdot}09$, 4 d.f., $P = 0{\cdot}54$; test for overall effect $z = 4{\cdot}20$, $P < 0{\cdot}001$



Reference	Year	Fibrin sealant: *n*	Fibrin sealant: Blood transfused (units)*	Control: *n*	Control: Blood transfused (units)*	Weight (%)	WMD (units)†
All studies‡							
Levy *et al*[29]	1999	29	0·2(0·5)	29	0·8(0·8)	58·9	− 0·62 (− 0·98, − 0·26)
Liu and Lu[33]	1993	20	7·9(5·4)	20	10·8(8·6)	0·4	− 2·92 (− 7·39, 1·55)
Noun *et al*[35]	1996	38	3·0(1·0)	44	3·5(1·2)	33·1	− 0·50 (− 0·98, − 0·02)
Uetsuji *et al.*[37]	1994	25	2·0(1·5)	39	2·3(2·5)	7·6	− 0·28 (− 1·27, 0·71)
Subtotal		112		132		100·0	− 0·56 (− 0·84, − 0·29)
In those patients transfused§							
Levy *et al*[29]	1999	5	1·2(0·4)	16	1·5(0·5)	100·0	− 0·30 (− 0·77, 0·17)

Fig. 2 Volume of allogeneic red cells transfused. *Values are mean(s.d.); †values are weighted mean difference (WMD) with 95 per cent confidence interval. FS, fibrin sealant. ‡Test for heterogeneity $\chi^2 = 1{\cdot}54$, 3 d.f., $P = 0{\cdot}67$; test for overall effect $z = 4{\cdot}03$, $P < 0{\cdot}001$. §Test for overall effect $z = 1{\cdot}25$, $P = 0{\cdot}2$

		Fibrin sealant		Control			
Reference	Year	*n*	Blood loss (ml)*	*n*	Blood loss (ml)*	Weight (%)	WMD (ml)†
Total intraoperative/postoperative blood loss‡							
Gasser *et al.*[34]	1983	44	60(68)	28	83(70)	26·5	– 22·94 (– 55·86, 9·98)
Levy *et al.*[29]	1999	29	1063(482)	29	1768(615)	8·5	– 705·00 (– 989·27, – 420·73)
Luke *et al.*[27]	1986	15	46(36)	15	249(278)	17·7	– 203·00 (– 344·82, – 61·18)
Noun *et al.*[35]	1996	35	242(249)	42	505(666)	11·9	– 263·00 (– 480·66, – 45·34)
Uetsuji *et al.*[37]	1994	25	1420(1101)	39	1626(1404)	2·4	– 206·00 (– 822·80, 410·80)
Vecsey *et al.*[38]	1980	20	18(21)	20	91(108)	25·7	– 72·70 (– 121·03, – 24·37)
Wurtz *et al.*[36]	1991	25	1170(568)	25	1093(588)	7·2	77·00 (– 243·47, 397·47)
Subtotal		193		198		100·0	– 151·68 (– 251·91, – 51·46)
Total postoperative blood loss measured via drains§							
Kohno *et al.*[31]	1992	31	164(147)	31	156(177)	28·2	8.00 (– 73·00, 89·00)
Levy *et al.*[29]	1999	29	360(288)	29	878(403)	25·9	– 518·00 (– 698·22, – 337·78)
Noun *et al.*[35]	1996	35	242(249)	42	505(666)	24·7	– 263·00 (– 480·66, –45·34)
Wurtz *et al.*[36]	1991	25	1170(568)	25	1093(588)	21·2	77·00 (– 243·47, 397·47)
Subtotal		120		127		100·0	– 180·59 (– 465·78, 104·60)
Estimated total postoperative blood loss¶							
Gasser *et al.*[34]	1983	44	60(68)	28	83(70)	33·8	– 22·94 (– 55·86, 9·98)
Levy *et al.*[29]	1999	29	1063(482)	29	1768(615)	11·0	– 705·00 (– 989·27, – 420·73)
Luke *et al.*[27]	1986	15	46(36)	15	249(27)	22·6	– 203·00 (– 344·82, – 61·18)
Vecsey *et al.*[38]	1980	20	18(21)	20	91(108)	32·7	– 72·70 (– 121·03, – 24·37)
Subtotal		108		92		100·0	– 154·67 (– 268·49, – 40·85)
Estimated total intraoperative blood loss**							
Jackson *et al.*[30]	1999	24	105(108)	23	94(121)	97·6	10·70 (– 54·98, 76·38)
Kohno *et al.*[31]	1992	31	1098(1453)	31	1027(951)	1·1	– 71·00 (– 540·31, 682·31)
Liu and Lui[33]	1993	20	3047(2142)	20	4052(3184)	0·1	– 1005·00 (– 2686·84, 676·84)
Uetsuji *et al.*[37]	1994	25	1420(1101)	39	1626(1404)	1·1	– 206·00 (– 822·80, 410·80)
Subtotal		100		113		100·0	7·47 (– 57·42, 72·36)



per cent c.i. −251·91 to −51·46) ml. Four trials reported blood loss in terms of total postoperative blood loss measured via drainage systems (WMD −180·59 (95 per cent c.i. −465·78 to 104·60) ml). Four trials reported total postoperative blood loss in terms of estimated blood loss (WMD − 154·67 (95 per cent c.i. −268·49 to −40·85) ml). For each of these subgroup analyses heterogeneity was statistically significant ($P < 0·001$). Total intraoperative blood loss, as reported by four trials, was unaffected by treatment (WMD 7·47 (95 per cent c.i. −57·42 to 72·36) ml). Heterogeneity between these trials was not statistically significant ($P = 0·59$).

Reoperation for bleeding

No episodes of reoperation for bleeding were reported by the two trials[28,32] that provided relevant data.

Mortality

Four trials[28–31] provided mortality data. Fibrin sealant use was not associated with any obvious change in mortality rate (RR 0·66 (95 per cent c.i. 0·18 to 2·38)) although the numbers of patients were too small to draw definite conclusions.

Length of hospital stay

Only two trials[35,36] reported data for length of hospital stay. Fibrin sealant use did not appear to have a significant impact on duration of hospital stay (WMD − 0·89 (95 per cent c.i. − 2·51 to 0·73) days).

Adverse events and other outcomes

Fibrin sealant use was not associated with an increase in any infection (RR 0·91 (95 per cent c.i. 0·37 to 2·25)), wound infection (RR 1·0 (95 per cent c.i. 0·19 to 5·26)) or pleural effusion (when used in cardiothoracic or hepatic surgery) (RR 0·25 (95 per cent c.i. 0·01 to 4·42)). However, the numbers of events were too small to draw firm conclusions. No trials reported data for deep venous thrombosis or wound dehiscence (outcomes selected *a priori*).

Discussion

The meta-analysis indicated that fibrin sealant use resulted in a mean reduction in blood loss of around 150 ml per patient, with most of the benefit being in the postoperative period. As a result, average transfusion requirements were reduced by around 0·6 units. In the authors' view these results must be interpreted with caution because of the generally poor quality of the trials. In particular, lack of blinding and the use of a subjective measure (the decision to transfuse) weakens the conclusions.

It is notable that even though blood loss differences were quite small, patients treated with fibrin sealants were transfused less frequently (60 per cent RR reduction). The trial conducted by Levy *et al.*[29] was the only study in which the reduction in mean total blood loss was both clinically and statistically significant (mean difference 705 ml; $P < 0·001$). For this trial, five of 29 patients in the fibrin sealant group were exposed to allogeneic red cells, compared with 16 of 29 in the control group. For the remaining four trials[28,34,35,38] that provided data for the proportion of patients exposed to allogeneic red cell transfusion the mean differences in total blood loss were modest, ranging from 23 to 263 ml. Despite this, the risk of exposure was reduced on average by a relative 57 per cent ($P < 0·001$).

A number of factors may account for the apparent discrepancy between blood loss and the frequency of blood transfusion. Blood loss may not have been measured accurately. The decision to transfuse blood was based on haemodynamic variables rather than blood loss. In the absence of transfusion protocols, blood transfusion practice may have been somewhat haphazard. As the majority of studies were unblinded, knowledge of the patient's treatment schedule may have reversed a transfusion decision, even when there was minimal blood loss. Although there appears to be some correlation between the volume of blood transfused (WMD −0·55 (95 per cent c.i. −0·83 to −0·28) units) and total blood loss (WMD −420·33 (95 per cent c.i. −760·30 to −80·36) ml) in trials that reported data for both these outcomes[29,35,37], this does not adequately explain the large reduction in the incidence of blood transfusion in patients treated with fibrin sealants.

The methodological quality of the trials was poor, with only three trials reporting the method used to generate allocation sequences. Full blinding of outcomes assessment did not occur in any study and only one trial[29] used a method of concealing treatment allocation that was judged to be adequate. All of the studies reviewed were small, with fewer than 50 participants in each arm. Reliance on small studies raises concerns about the effects of publication bias. The

Fig. 3 Volume of intraoperative/postoperative blood loss. *Values are mean(s.d.), †values are weighted mean difference (WMD) with 95 per cent confidence interval. FS, fibrin sealant. ‡Test for heterogeneity $\chi^2 = 32·79$, 6 d.f., $P < 0·001$; test for overall effect $z = 2·97$, $P = 0·003$. §Test for heterogeneity $\chi^2 = 30·76$, 3 d.f., $P < 0·001$; test for overall effect $z = 1·24$, $P = 0·2$. ¶Test for heterogeneity $\chi^2 = 28·31$, 3 d.f., $P < 0·001$; test for overall effect $z = 2·66$, $P = 0·008$. **Test for heterogeneity $\chi^2 = 1·90$, 3 d.f., $P = 0·59$; test for overall effect $z = 0·23$, $P = 0·8$

degree to which publication bias may have impacted on the overall results of this review is difficult to determine accurately as the small number of trials hampered any formal assessment using funnel plotting techniques. As the presence of publication bias cannot be discounted, the results need to be viewed with caution.

The most significant degree of heterogeneity in these trials related to intraoperative/postoperative blood loss. The observed variation was generally in terms of size, not the direction of effect. To explain the variation in the size of treatment effect a number of factors was examined, including the type of surgery, the timing of blood loss measurement and the methods used to measure blood loss. Most of the observed heterogeneity seemed to be explained by the type of surgery. Subgroup analysis by the type of fibrin sealant was constrained by the small number of trials.

The International Study of Perioperative Transfusion (a ten-country study of evidence, attitudes and practices relating to the use of alternatives to perioperative allogeneic blood transfusion) has generated a number of systematic reviews that examined the efficacy and safety of various technologies employed to minimize the need for perioperative allogeneic blood transfusion[20,39–41]. Among the technologies studied were antifibrinolytic drugs (aprotinin, tranexamic acid and epsilon aminocaproic acid), desmopressin (deamino-8-D-arginine vasopressin), autologous blood donation by preoperative deposit, acute normovolaemic haemodilution and cell salvage. Findings from these studies indicate that preoperative deposit, haemodilution and cell salvage (techniques for reinfusing a patient's own blood, not for reducing blood loss) are associated with only small benefits in terms of reducing the need for allogeneic blood. However, the use of technologies intended to reduce surgical blood loss, such as aprotinin and tranexamic acid, are associated with significant benefits in terms of reducing the need for allogeneic blood transfusion and the need for reoperation owing to continued bleeding[20]. The data considered here are insufficient to conclude whether fibrin sealants are similarly effective.

The studies reviewed here provided inadequate data for firm conclusions to be drawn about the impact of fibrin sealant use on clinically important endpoints. Furthermore, the generalizability of the results across different clinical settings and different fibrin sealants is uncertain. In such circumstances large randomized controlled trials with clinical outcomes are recommended.

Acknowledgements

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Randomized clinical trial comparing loop ileostomy and loop transverse colostomy for faecal diversion following total mesorectal excision

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Background: The aim of this study was to compare loop ileostomy and loop transverse colostomy as the preferred mode of faecal diversion following low anterior resection with total mesorectal excision for rectal cancer.

Methods: Patients who required proximal diversion after low anterior resection with total mesorectal excision were randomized to have either a loop ileostomy or a loop transverse colostomy. Postoperative morbidity, stoma-related problems and morbidity following closure were compared.

Results: From April 1999 to November 2000, 42 patients had a loop ileostomy and 38 had a loop transverse colostomy constructed following low anterior resection. Postoperative intestinal obstruction and prolonged ileus occurred more commonly in patients with an ileostomy ($P = 0{\cdot}037$). There was no difference in time to resumption of diet, length of hospital stay following stoma closure and incidence of stoma-related complications after discharge from hospital. A total of seven patients had intestinal obstruction from the time of stoma creation to stoma closure (six following ileostomy and one following colostomy; $P = 0{\cdot}01$).

Conclusion: Intestinal obstruction and ileus are more common after loop ileostomy than loop colostomy. Loop transverse colostomy should be recommended as the preferred method of proximal faecal diversion.

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Introduction

Anastomotic leakage is a serious postoperative complication following colorectal surgery. The incidence is particularly high when an anastomosis involves the distal rectum or anal canal[1,2]. Proximal faecal diversion reduces the incidence of clinical anastomotic leakage following low anterior resection with total mesorectal excision[3]. Although use of proximal faecal diversion to prevent leakage at rectal anastomoses remains controversial[2,4], most authors agree that a defunctioning stoma can reduce the risk of sepsis resulting from anastomotic leakage[5,6].

Proximal diversion of a distal rectal anastomosis can be achieved by either a loop colostomy or a loop ileostomy. It remains controversial whether a loop ileostomy or a loop transverse colostomy is a better form of faecal diversion. This randomized controlled trial compared loop ileostomy and loop transverse colostomy to determine the optimal mode of faecal diversion following low anterior resection with total mesorectal excision for rectal cancer.

Patients and methods

Patients who underwent low anterior resection for rectal cancer within 12 cm from the anal verge were eligible for inclusion in the study, which took place from April 1999 to November 2000. Informed consent was obtained from patients before the operation. The stoma therapist marked both the loop ileostomy site and the loop transverse colostomy site before surgery. During the study period all patients treated with total mesorectal excision with an anastomosis at or below 5 cm from the anal verge had a diverting stoma. Patients with proximal rectal cancer in whom the mesorectum was transected and a higher rectal anastomosis constructed (more than 5 cm from anal verge) did not have faecal diversion. The decision to create a diverting stoma and randomization of patients was made on completion of the colorectal or coloanal anastomosis.

In patients randomized to have a loop ileostomy, the loop of ileum destined for the ileostomy was brought out without tension through the abdominal wall at the premarked stoma site. The distal limb of the loop was marked with a suture. A transverse incision was made involving the antimesenteric two-thirds of the ileum and a 1·5–2·0-cm spout constructed by everting the afferent limb. The loop was not rotated and the afferent limb constituted the superior aspect of the stoma.

In patients randomized to have a loop transverse colostomy, the stoma was created in the transverse colon to the right of the middle colic vessels. The mesentery adjacent to the bowel wall was incised for the passage of a nelaton catheter, used to bring the transverse colon out through the premarked stoma site. The catheter was also used to support the colostomy in the early postoperative period and was removed 1 week after surgery. An incision was made along one of the taenia coli and the colostomy was matured with catgut sutures.

A contrast enema was performed before closure of the stoma to ensure the integrity of the anastomosis. Mechanical bowel preparation with polyethylene glycol solution was given to the patients the day before closure of the transverse colostomy. No bowel preparation was administered before closure of an ileostomy. Perioperative antibiotics were given at the time of induction of anaesthesia. A circumstomal incision was used for stoma closure. The loop of colon or ileum was freed by sharp dissection. Primary closure of the anterior colonic wall was performed for the loop transverse colostomies while resection and anastomosis was the procedure for closure of the loop ileostomies. The abdominal wall was closed with interrupted polydioxanone sutures and the skin was closed primarily.

Demographic data, details of the operation, postoperative complications and follow-up data formed part of the prospective database of patients with rectal cancer. Prolonged postoperative ileus was defined as the inability to tolerate diet 5 days after surgery in the absence of active bowel sounds with the need for nasogastric decompression. Data on stoma function and stoma-related morbidity were collected.

Sample size calculation

The authors had shown previously that 15 per cent of patients who had a loop ileostomy for faecal diversion following rectal resection developed small bowel obstruction. It was assumed that obstruction was due mainly to the presence of a loop ileostomy which would not have occurred in case of a loop transverse colostomy. To reduce the obstruction rate by 15 per cent, it was estimated that a sample size of 36 patients in each group would be required to achieve a type I error of 0·05 with a power of 0·8. In calculation of the sample size, it was assumed that 10 per cent of the patients would not undergo stoma closure. Therefore it was planned to recruit 80 patients.

Statistical analysis

Nominal variables were compared with the χ^2 test or Fisher's exact test as appropriate. Continuous variables were presented as means and compared with Student's *t* test. Non-parametric data were presented as median values and compared with the Mann–Whitney *U* test. $P < 0{\cdot}05$ was considered statistically significant.

Results

Eighty patients, 31 women and 49 men, were randomly assigned to have a loop ileostomy (42) or a loop transverse colostomy (38). The mean age was 66·5 (range 38–87) years. There were no significant differences in demographic data, tumour characteristics or operative details between the two groups (*Table 1*).

There were two intraoperative complications, one splenic injury and one ureteric injury during concomitant total hysterectomy and bilateral oophorectomy. Non-stoma-related complications occurred in 13 patients in the ileostomy group and in eight patients in the transverse colostomy group ($P = 0{\cdot}45$). Clinical anastomotic leakage occurred in four patients, two in each group. Laparotomy and peritoneal lavage was required in one patient while the other patients were managed without surgery. As regards stoma-related morbidity, three patients developed intestinal obstruction and four developed ileus in the ileostomy group while prolonged ileus occurred in only one patient in the colostomy group ($P = 0{\cdot}037$). One patient required surgery; the obstruction was due to a kink in the ileum just proximal to the ileostomy site.

Recovery of bowel function and time to resumption of diet after the initial operation are shown in *Table 1*. Bowel function returned and a fluid diet was resumed more quickly in patients with a loop ileostomy. However, the time to resumption of solid diet and the length of hospital stay were no different.

Two patients in the ileostomy group died in the early postoperative period because of bowel ischaemia. Another patient developed gastrointestinal bleeding from a duodenal ulcer, resulting in hypotension and ischaemic injury to the colon. Emergency vagotomy and pyloroplasty with takedown of the anastomosis was performed. These three patients were excluded from the analysis of stoma-related complications after discharge from hospital.

Table 2 shows the stoma complications that occurred before closure. There were no differences in the number of complications between the two groups. The most common

Table 1 Comparison of patients with loop ileostomy and loop transverse colostomy

	Loop ileostomy (*n* = 42)	Loop transverse colostomy (*n* = 38)	*P*
Sex ratio (F : M)	16 : 26	15 : 23	0·89
Mean age (years)	65·2	67·8	0·28
Mean tumour level (cm)*	6·8	6·4	0·36
Mean level of anastomosis (cm)*	3·7	3·3	0·17
Anastomosis type			0·60
Double stapled	41	36	
Hand-sewn coloanal	1	2	
Additional organs resected	3	2	1·00
Mean blood loss (ml)	451	521	0·38
Mean duration of operation (min)	163	158	0·59
Laparoscopically assisted	3	3	1·00
Stage†			0·12
I	7	2	
II	21	14	
III	12	19	
IV	2	3	
Median time to stoma output (days)	3	4	0·001
Median time to fluid diet (days)	2	4	0·04
Median time to solid diet (days)	4	4	0·80
Median length of hospital stay (days)	9	9	0·74

*Above anal verge. †Stage according to American Joint Committee on Cancer

Table 2 Stoma-related complications during follow-up

	Loop ileostomy (*n* = 39)	Loop transverse colostomy (*n* = 38)
Prolapse	0	3
Retraction	0	0
Skin irritation	4	7
Parastomal hernia	1	0
High output	1	0
Incomplete diversion	0	1
Total	6	11*

*P = 0·16 *versus* loop colostomy (Fisher's exact test)

complications were minor skin problems, which occurred in 11 patients. No operations were needed to deal with the stoma complications.

One patient died from pneumonia before closure of the stoma. Three patients preferred not to the have the stoma closed, two because of irresectable liver metastases. Stoma closure was therefore performed in 73 patients (35 ileostomies and 38 colostomies). The median time to closure was 183 days for patients with an ileostomy and 180 days for those with a loop colostomy (P = 0·63). The duration of operation, time to recovery of bowel function and time to resumption of diet are shown in *Table 3*. The duration of operation was shorter in the colostomy group. Patients in the ileostomy group had earlier return of bowel function. However, there were no differences in the time to resume fluid and solid diet or length of hospital stay.

No death resulted from stoma closure. The morbidity of stoma closure is shown in *Table 3*. In the ileostomy group three patients had intestinal obstruction, one of whom required operative treatment. One patient in the loop transverse colostomy group developed intestinal obstruction following closure of the stoma which resolved with conservative treatment.

Overall, six of 35 patients who had an ileostomy that was ultimately closed developed intestinal obstruction. Obstruction occurred in one of the 38 patients who had a loop transverse colostomy (P = 0·01).

Discussion

Total mesorectal excision with sphincter-preserving surgery has become the treatment of choice for cancers of the middle and lower third of the rectum[7,8]. However, the anastomotic leakage rate has been shown to be particularly high following distal colorectal or coloanal anastomosis[2,9,10]. A few studies have shown lower anastomotic leakage rates in patients in whom there is proximal faecal diversion[3,11].

The optimal mode of faecal diversion is debatable as it can be achieved by either a loop transverse colostomy or a loop ileostomy. Initially used for defunctioning the colon in patients with fulminant ulcerative colitis[12,13], loop ileos-

Table 3 Events after stoma closure

	Loop ileostomy (*n* = 35)	Loop transverse colostomy (*n* = 38)	*P*
Mean duration of operation (min)	61	51	0·008
Median time to first defaecation (days)	2	3	0·003
Median time to fluid diet (days)	2	2	0·49
Median time to solid diet (days)	3	3	0·69
Surgical complications			
Wound infection	1	2	
Enterocutaneous fistula	1	0	
Intestinal obstruction	3	1	
Total no. of patients	4*	3	0·70
Medical complications	0	2	0·49
Pneumonia	0	1	
Arrhythmia	0	1	
Median hospital stay (days)	5	6	0·64

*One patient had intestinal obstruction and wound infection

tomies have been used increasingly to provide proximal faecal diversion for distal colonic or rectal anastomoses[14]. Four randomized trials have compared loop colostomy and loop ileostomy[15–18]. Three of these studies recommended loop ileostomy[15,17,18] while one recommended loop transverse colostomy[16]. Three of the earlier studies included patients with various colorectal pathologies as well as anastomoses at different levels in the colon and rectum[15–17].

The recent study by Edwards *et al.*[18], which is similar to the present study, included only patients who had total mesorectal excision for rectal cancer. They found more stoma-related complications following closure of a loop colostomy, although colostomy closure was technically easier. The most common complication was incisional hernia, which occurred during follow-up. In the present study, more emphasis was placed on early postoperative morbidity and stoma-related morbidity. Although loop ileostomies started to function earlier than loop transverse colostomies, this did not translate into earlier resumption of a solid diet or a shorter hospital stay. A higher incidence of postoperative ileus and intestinal obstruction was found in the ileostomy group after the initial operation. The results are similar to those of the randomized controlled trial by Gooszen *et al.*[16], who reported more postoperative complications in patients with a loop ileostomy. The presence of a loop ileostomy in the infracolic compartment is more likely to create adhesion, twisting or herniation of the small bowel, leading to postoperative obstruction.

Stoma complications during follow-up were similar in the two study groups and were not serious compared with those reported in other studies[16]. The most common problems involved the peristomal skin and occurred at a similar rate in both groups. Prolapse of the stoma occurred in three patients with a loop transverse colostomy. This incidence is similar to that reported by Edwards *et al.*[18] and is much lower than the incidence reported by Gooszen and colleagues[16]. The fascial defect created for the colostomy was not large and was similar to that used for loop ileostomy. A flush colostomy was created without bringing the whole transverse colon out beyond skin level. This may account for the relatively low incidence of prolapse in the colostomy group.

The incidence of septic complications following closure of the stoma was similar in both groups. Intestinal obstruction occurred in three patients following closure of a loop ileostomy and resulted in one reoperation. This finding is similar to that in a previous review when loop ileostomy was the preferred defunctioning stoma[19]. Furthermore, the incidence of intestinal obstruction from the time of creation of the stoma to the postoperative period of closure was significantly higher in the ileostomy group.

Intestinal obstruction is a well recognized complication associated with ileostomy although its incidence following rectal cancer surgery is less well documented. Metcalf *et al.*[20] reported an incidence of intestinal obstruction of 6·4 and 14·8 per cent before and after closure of loop ileostomies respectively in patients with an ileoanal pouch anastomosis. The presence of a loop ileostomy may increase the chance of twisting the small bowel and the formation of adhesions adjacent to the stoma. Francois *et al.*[21] reported that temporary ileostomy was responsible for two-thirds of the cases of postoperative obstruction in patients with restorative proctocolectomy.

Intestinal obstruction following loop ileostomy closure has also been well studied[22–24]. Closure of a loop ileostomy is a more difficult operation than closure of a loop colostomy[18]. In the present study, it took longer to close a loop ileostomy. Recent reports show that the method of closure has an important impact on subsequent intestinal obstruction[22,25]. Hasegawa *et al.*[25] showed that stapled anastomosis during closure of an ileostomy was associated with a lower incidence of postoperative intestinal obstruction in a randomized controlled trial, while Phang *et al.*[22] found that enterotomy suture was associated with the lowest incidence of postoperative intestinal obstruction. Further studies are required to confirm their findings.

In this randomized controlled trial, a higher incidence of postoperative ileus and intestinal obstruction was found in patients in whom a loop ileostomy was used for proximal faecal diversion. Loop ileostomy was found to be associated with a higher incidence of intestinal obstruction from the time of its creation to the postoperative period of closure. As other stoma-related complications were comparable in the two groups, a loop transverse colostomy appears to be the optimal form of faecal diversion for a distal rectal or anal anastomosis following total mesorectal excision.

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Vascular Surgical Society of Great Britain and Ireland

The 35th Annual Meeting of the Vascular Surgical Society of Great Britain and Ireland was held in Brighton, UK on 7–9 November 2001 under the Presidency of Mr R. N. Baird. Abstracts of the presentations given at the meeting were published in the April issue of the *BJS*. The following articles are based on presentations from the Brighton meeting and were accepted for publication after peer review by the Journal's referees.

Vascular Surgical Society

Effect of venous ulcer exudates on angiogenesis *in vitro*

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Background: Angiogenesis, the formation of new from existing capillaries, is an important mechanism in venous ulcer healing. The aim of this study was to determine whether venous leg ulcer wound exudates stimulate or inhibit angiogenesis.

Methods: Fluid exudate was obtained from 16 venous ulcers over a 4-h interval. Five of the ulcers had not healed after more than 1 year of compression bandaging, and five were rapidly healing ulcers. As a control, acute wound fluids were collected from subcutaneous drains in seven patients. Vascular endothelial growth factor (VEGF) at 2 ng/ml acted as a positive control. Tubules stained with an antiendothelial antibody were quantified using an image analysis system. The extent of angiogenesis was expressed as the ratio of the mean tubule length in the test wells over that in blank control wells.

Results: Venous ulcer exudates significantly inhibited angiogenesis (mean (95 per cent confidence interval) 0·72 (0·48 to 0·96)) compared with acute wound fluids (2·48 (0·86 to 4·10)) ($P < 0·002$) and VEGF (1·47 (1·32 to 1·61)) ($P = 0·01$). Exudates from the five non-healing venous ulcers inhibited angiogenesis (0·31 (0·15 to 0·46)) significantly more than exudates from the five rapidly healing venous ulcers (0·93 (0·21 to 1·65)) ($P = 0·03$).

Conclusion: Fluid exudate from venous ulcers, in particular those that healed slowly, inhibited experimental angiogenesis in this study.

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Introduction

Venous ulcers are a common problem, having a lifetime prevalence of between 1 and 3 per cent in western Europe[1–3]. Ulcer treatment is costly in terms of dressings, bandages and nursing time[4]. Between 30 and 60 per cent of venous ulcers heal within 3 months of treatment with compression bandaging[5,6], but 20 per cent persist beyond 1 year and may require excision and skin grafting. Some venous ulcers never heal despite adequate treatment and, anecdotally, these ulcers often have white and fibrous bases[7].

Angiogenesis, the formation of new from existing capillaries, is a part of normal wound healing. The microcirculation is known to be abnormal in venous disease. Histological studies have shown that the local capillaries in lipodermatosclerotic skin are abnormally tortuous and leaky[8], with disrupted basement membranes[9]. Capillary density correlates with disease severity[8]. Capillary proliferation has been associated with increased staining for vascular endothelial growth factor (VEGF)[10], the most potent angiogenic growth factor, which also induces capillary permeability. Increased VEGF levels in venous

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The oral presentation based on this manuscript won the *BJS* prize at the above meeting blood have been reported in patients with chronic venous insufficiency[11]. Capillary microscopy has suggested that there are fewer capillaries in lipodermatosclerotic skin than in normal skin, although they are longer and more coiled[12]. An attempt has been made to link the number of vessels at the edge of a venous ulcer seen by capillary microscopy with the rate of healing, but the study was too small to confirm a significant link[13].

The proteolytic milieu in the base of a venous ulcer may degrade any growth factors that are produced, adversely affecting angiogenesis and delaying wound healing[14]. Angiogenic inhibitors such as angiostatin, produced after plasminogen is cleaved to form plasmin, may also delay healing[15].

There is a clear indication that angiogenesis is abnormal in patients with venous disease. In this study, the effects of acute wound fluid exudates from a variety of venous ulcers were examined using an *in vitro* angiogenesis assay.

Patients and methods

Patients

Sixteen patients with a typical venous ulcer, i.e. associated with varicose veins, venous eczema, lipodermatosclerosis and venous reflux on duplex imaging, were recruited from the venous ulcer clinic. All patients had palpable pedal pulses and none had diabetes. Patients were recruited on first attendance to the clinic, at which three-layer paste or four-layer compression bandaging was commenced. Ulcers had been present for varying time intervals and had various treatments before recruitment. All patients were followed until ulcer healing, or for at least 1 year. A slow-healing ulcer was defined as one that persisted for more than 1 year. Ulcers that healed within 3 months were considered to be rapid healing.

Seven patients who required a closed suction drain after surgery were recruited as controls for the study. There were four women who had undergone breast surgery, and three men who had had carotid endarterectomy, thyroidectomy or lymphoedema reduction.

Collection of samples

Venous ulcers were cleansed and covered with an occlusive, adhesive dressing (OpSite™; Smith and Nephew Medical, Hull, UK), using ether to improve adhesion. After 4 h, the collected fluid was aspirated from beneath the dressing using a 5-ml syringe and 21-G needle. Acute wound fluids were collected from the drain bottles on the second day after operation.

Immediately after collection, all fluids were spun at 13 000*g* for 10 min, and the supernatant was snap frozen and stored at − 70°C until required. Just before assay, wound fluids were thawed, diluted to 0·5 per cent by volume in endothelial growth medium supplemented with 2 per cent fetal calf serum (TCS CellWorks, Buckingham, UK), and filtered through a 0·22-μm filter to remove bacterial contamination.

In vitro angiogenesis kit

A commercially available angiogenesis kit was used (TCS CellWorks). This consists of islands of human umbilical vein endothelial cells co-cultured with dermal fibroblasts on flat-bottomed 24-well plates. After incubation for 11–14 days, cord-like structures appear, which have been demonstrated by confocal and electron microscopy to be tubular and have a lumen[16].

Wound fluids were assayed by a blinded investigator, in triplicate, at a fixed concentration of 0·5 per cent by volume. Culture medium containing wound fluid was replenished after 3, 6 and 9 days. A blank control (culture medium only) and a positive control (VEGF 2 ng/ml) were used on each plate.

On day 12, the medium was aspirated carefully and the plates were fixed with cold 70 per cent ethanol, and stained for CD31 (platelet endothelial cell adhesion molecule 1) using mouse antihuman CD31 as the primary antibody, goat antimouse immunoglobulin G–alkaline phosphatase conjugate as the secondary antibody and 5′-bromo-4-chloro-3-inodylphosphate–nitroblue tetrazolium tablets (Sigma®, Bookham, UK) as substrate. This reaction stained the tubules a dark purple colour.

Each well was photographed using a digital camera (Coolpix 990; Nikon UK, Kingston, UK) on an inverted microscope at 40× magnification. Five images were taken for each well in a grid-like fashion at a resolution of 2048 × 1536 pixels. A background image of an empty well was taken and all images were subtracted from this in order to correct for differences in light and shadow, using an image analysis software package (Image-Pro® Plus, Silver Spring, Maryland, USA). The number of pixels stained dark in the image was then calculated to provide a numerical value for the degree of tubule formation.

Because of the variability inherent in a biological assay, the pixel value for each treatment on each plate was divided by the blank controls on the same plate to allow comparison between plates.

Values in text are mean (95 per cent confidence interval). Groups were compared using the Mann–Whitney *U* test.

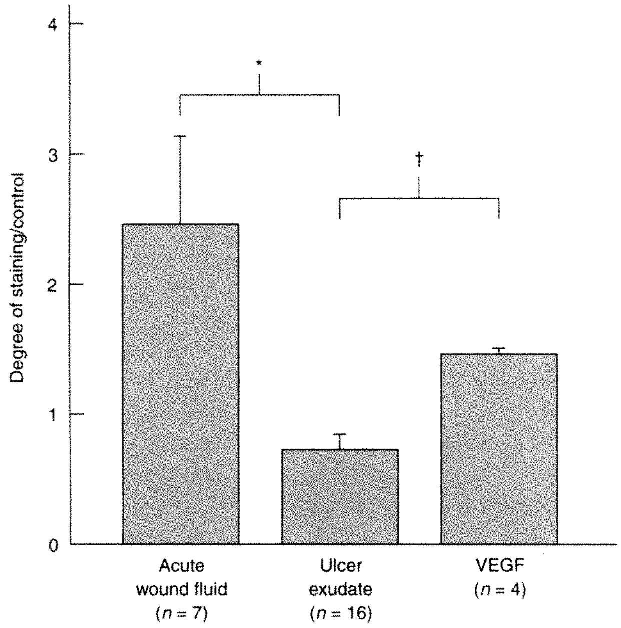

Fig. 1 Effect of acute wound fluid, venous ulcer exudate and vascular endothelial growth factor (VEGF) on *in vitro* angiogenesis. Bars and error bars represent mean and standard error of the mean. $^*P < 0{\cdot}002$, $^†P = 0{\cdot}01$ (Mann–Whitney *U* test)

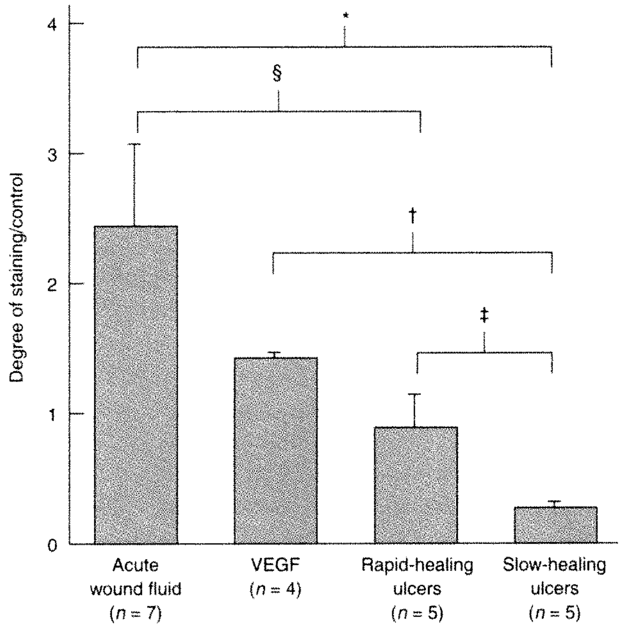

Fig. 2 Comparison of the effect of acute wound fluid, vascular endothelial growth factor (VEGF), and exudate from rapid- and slow-healing venous ulcers on *in vitro* angiogenesis. Bars and error bars represent means and standard error of the mean. $^*P < 0{\cdot}003$, $^†P < 0{\cdot}02$, $^‡P = 0{\cdot}03$, $^§P < 0{\cdot}05$ (Mann–Whitney *U* test)

Results

The 16 patients with a venous ulcer included ten men and six women of median age 64 (range 36–80) years. Five ulcers were less than 10 cm^2 in diameter, eight were between 10 and 100 cm^2, and three were greater than 100 cm^2. One patient died. Five slow-healing ulcers did not heal within 1 year and five rapid-healing ulcers healed within 3 months; the others healed by 3–6 months ($n = 3$) or 6–12 months ($n = 2$).

Venous ulcer exudates inhibited angiogenesis (0·72 (0·48 to 0·96); $n = 16$) compared with acute wound fluids (2·48 (0·86 to 4·10); $n = 7$) ($P < 0{\cdot}002$) (*Fig. 1*). A similar difference was observed when the ulcer fluids were compared with VEGF-treated wells (1·47 (1·32 to 1·61); $n = 4$) ($P = 0{\cdot}01$) (*Fig. 1*). There was no difference in angiogenesis between acute wound fluid and VEGF treatment.

The exudates from the five slow-healing venous ulcers inhibited angiogenesis (0·31 (0·15 to 0·46); $n = 5$) significantly more than exudates from five patients with rapidly healing ulcers (0·93 (0·21 to 1·65); $n = 5$) ($P = 0{\cdot}03$) (*Fig. 2*). There was no difference between the degree of tubule formation in wells treated with exudates from the venous ulcer group as a whole (0·75 (0·49 to 1·00); $n = 16$) and those treated with exudates from rapidly healing ulcers (0·93 (0·21 to 1·65); $n = 5$).

Discussion

Studies have shown that acute wound fluids (from split skin graft donor sites or subcutaneous drains) contain angiogenic stimulators[17–19]. The results presented in this study confirm these findings, as addition of acute wound fluid to the angiogenesis assay resulted in a 2·5-fold increase in tubule formation. One previous study that investigated the effects of venous ulcer exudates on endothelial cell mitogenesis used a proliferation assay to demonstrate that pooled venous ulcer exudates, at a concentration of 2 per cent, failed to stimulate or inhibit the growth of human dermal microvascular endothelial cells[20]. The angiogenesis assay (involving proliferation, migration and adhesion) used in the present study showed that venous ulcer exudates significantly reduced tubule formation. This inhibition was more pronounced in ulcers that healed slowly.

The cause of the inhibition of angiogenesis by ulcer exudates in this study is not known. It is possible that the exudates caused the endothelial cells to detach from the plates rather than inhibiting tubule formation, which may be a consequence of the proteolytic nature of these fluids. This seems unlikely, however, as the exudates were diluted to 0·5 per cent. The authors have observed in mitogenesis assays that this concentration did not detach cells from the plate.

One possible explanation is that there is an inhibitor present in these fluids. This agent may be an enzyme that inhibits angiogenesis by degrading growth factors required for capillary budding and growth. Levels of urokinase-type plasminogen activator and the product of its activity, the serine protease plasmin, have been found to be raised in ulcer tissue biopsies and exudates[21,22]. It has previously been demonstrated that the activity of this enzyme was significantly higher in biopsies from slow-healing venous ulcers[23]. It has been suggested that plasmin is the enzyme responsible for the cleavage of growth factors in ulcer exudate[14]. These factors may be degraded in such a way as to inactivate them without affecting their levels as measured by antigen-based assays[14]. This might explain the findings of Harris *et al.*[24], who showed that chronic venous ulcer exudates contained collagenase activity, but could find no difference between levels of cytokines in healing and non-healing ulcers.

There are low levels of plasminogen and high levels of plasmin in ulcer exudates. Angiostatin is a cleavage product of plasminogen when plasmin is formed and has potent antiangiogenic activity[15,25]. This molecule is therefore a potential candidate as a specific angiogenic inhibitor in ulcer exudates.

The angiogenesis kit used in this study is the only commercially available *in vitro* angiogenesis kit in the UK. The main criticism of this kit, however, is that the human endothelial cells used are neonatal macrovascular cells, rather than adult dermal microvascular cells that are found in the skin of patients with venous ulcers. The kit used in this study had a low intra-assay variability but a high interassay variability, making comparisons between different batches difficult; this problem was addressed by expressing the result for each well in relation to the corresponding blank control for that plate.

Ulcer exudates are complex solutions that are difficult to standardize and study[26]. The exudates used in this study were diluted by volume rather than protein concentration, as it was felt that this more accurately reflects the wound environment. Ulcer exudates were collected over 4 h and frozen immediately, to prevent possible degradation of growth factors by proteolytic enzymes. This produced small volumes of exudate that precluded an attempt to demonstrate a dose–response relationship.

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Vascular Surgical Society

A meta-analysis of 50 years of ruptured abdominal aortic aneurysm repair

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Background: Operative repair of ruptured abdominal aortic aneurysm (RAAA) is associated with a high mortality rate but reported figures vary widely. The aim of this study was to estimate the operative mortality of RAAA repair and determine how it has changed over time.
Methods: A meta-analysis of all English language literature quoting figures for operative mortality of RAAA repair.
Results: The pooled estimate for the overall operative mortality rate of RAAA repair from 1955 to 1998 was 48 (95 per cent confidence interval 46 to 50) per cent. Meta-regression analysis of operative mortality over time demonstrated a constant reduction of approximately 3·5 per cent per decade (1954–1997) with an operative mortality rate estimate for the year 2000 of 41 per cent. Seventy-seven studies reported intraoperative mortality but, while this appears to have remained constant over time, there was evidence of the presence of publication bias in the subgroup of papers reporting this outcome. There was no evidence of publication bias for the overall operative mortality outcome.
Conclusion: Contrary to the conclusion of recent studies, this paper demonstrates a gradual reduction with time in the operative mortality rate of RAAA repair.

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Introduction

In 1989 it was reported that the true number of deaths due to ruptured abdominal aortic aneurysm (RAAA) in England and Wales was approximately 7500 per annum[1]. Since the mortality rate associated with RAAA has increased steadily over the past two decades (*Fig. 1*) the annual figure is now likely to be considerably higher. So RAAA is a significant cause of mortality in the UK, accounting for a similar number of deaths as gastric, oesophageal and prostatic malignancies[3]. Despite this, the importance and implications of RAAA remain largely unacknowledged by the medical establishment, and the majority of the lay public are unaware of the existence of the disease.

The only curative treatment for RAAA is emergency operation and there is a high operative mortality rate. Reported rates vary from as low as 15 per cent[4] to as high as 90 per cent[5] but are usually quoted as approximately 45 per cent[6–9]. With surgeons' increased familiarity with the operative procedure and improvements in critical care, it might be expected that this figure should be improving, but it appears to have remained unchanged since earlier reports[10,11].

The aim of this study was to estimate the operative mortality rate of RAAA repair, quantify how this has changed over time, and examine changes in the demographic features of patients undergoing RAAA repair, by performing a meta-analysis of the relevant English language literature. This article was prepared according to previously published guidelines for reporting meta-analyses of observational studies[12].

Methods

Search strategy

Literature searches of the Medline (January 1966 to November 2000) and Embase (January 1980 to November 2000) electronic databases were performed by one of the authors (M.J.B.). The Ovid search engine (version 7.8; Ovid Technologies, New York, USA) was employed. The following search strategies were each used on both

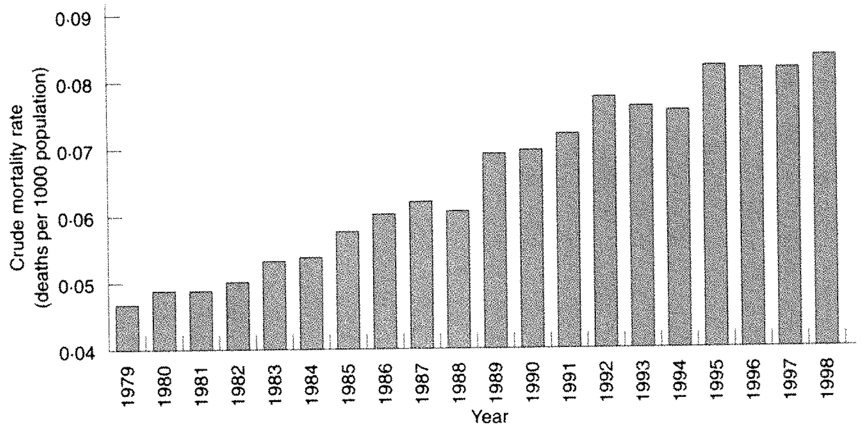

Fig. 1 Deaths due to ruptured abdominal aortic aneurysm in England and Wales 1979–1998[2]

databases: (a) 'aortic aneurysm, abdominal *and* mortality (textword) *and* aortic rupture/mortality', (b) 'aortic aneurysm, abdominal/mortality, complications *and* [aortic rupture *or* rupture (textword) *or* rupture, spontaneous]', (c) 'aortic rupture *and* [aortic aneurysm, abdominal *or* abdominal aortic aneurysm (textword)]'. In addition, manual searching of reference lists from articles retrieved by electronic searching was used to identify those articles published before the inception of these databases and any missed by the electronic searches. Articles retrieved were restricted to those published in English.

Study selection

All articles that gave figures for total number of patients undergoing surgery for RAAA and the total number of those who died were included in the analysis. Articles were not restricted to any particular operative technique; transperitoneal, retroperitoneal and non-inlay were all included. Studies of all types of design were included, both prospective and retrospective observational studies, and experimental studies. For experimental studies, data from both the control and intervention arms of the study were compiled and included as a single study.

Articles were excluded if they did not explicitly define RAAA as the presence of blood outside the aortic wall at laparotomy. This was to exclude papers that included patients undergoing emergency or urgent repair of symptomatic non-ruptured AAA in their figures, since this diagnosis is known to be associated with a lower mortality rate than RAAA[9,13]. Articles were rejected if they described only selected groups of patients (such as octogenarians), were single case reports or did not include intraoperative deaths in their figure for overall deaths. Papers selected from English language abstracts that were subsequently found to be written in other languages were also rejected.

Data extraction

All articles were reviewed by one of the authors (M.J.B.). For all studies the total number of patients undergoing surgery, the total number of those who died, and the mid-timepoint of the study (the date half-way through the study time period) were recorded. Where mortality figures were quoted as percentages actual numbers were calculated from the data provided. The definition of operative mortality used in each study was recorded (in-hospital or 30 day). In addition, when given, the number of patients who died during surgery, the gender ratio of the study population (proportion of women in the study group), the average age of the study population (mean or median) and the number of patients denied surgery by the operating surgeons were recorded. The number of patients refused surgery was not included in the figure for total number of patients undergoing surgery. Where both the mean and median ages were given median age was recorded.

Validity assessment

Ascertaining the validity and quality of largely non-comparative observational studies in the context of meta-analyses is not straightforward as there are no quality scales or checklists developed specifically for such studies. For this reason, individual study characteristics perceived as potentially related to the validity and quality of these particular studies were examined[14]. Whether each study was a multicentre or single-centre study, whether the data on patient numbers had been obtained via the use of coded computerized records and the level of evidence according to the definitions of Blankensteijn *et al.*[15] were all recorded.

Quantitative data synthesis

Separate meta-analyses were performed for operative mortality and intraoperative mortality outcomes. These were both performed using random-effects models on a log odds outcome scale. The presence of between-studies heterogeneity was assessed using the standard χ^2 test (test statistic denoted as Q in the results). Results were transformed to the proportion scale to ease interpretability[14]. In an attempt to explain any between-study heterogeneity, additional meta-regression analyses were performed to explore the effect of time (using the co-variate midpoint of study), patient age (using median/mean patient age), gender (using the co-variate proportion of population female) and number of patients turned down for surgery. A mixed model, again using the log odds outcome scale, was utilized for this purpose[16].

Sensitivity analysis was performed, stratifying analyses by the measures of quality identified in the previous section, to examine whether there was systematic variation in results across these quality components. Funnel plots were examined to assess whether publication bias was likely to be a problem, and the method of trim and fill[17,18], which assesses how many studies are likely to be missing and the potential impact of their exclusion on the analysis, applied to both outcomes. All statistical analyses were carried out using Stata Statistical Software, release 7.0 (Stata, College Station, Texas, USA)

Results

Study flow (*Fig. 2*)

A total of 517 articles were identified, 456 by computerized searches and 61 by manual searching of references. Fifty-seven were duplicates owing to the use of electronic searching of both the Medline and Embase databases. One hundred and twenty-one were rejected on the basis of electronic examination of their abstracts, from which it was clear that they did not concern RAAA. All articles without electronically available abstracts were retained. The remaining 340 articles were obtained via local library collections, The British Medical Association Library and The British Library. Three articles could not be obtained from any of the above sources[19–21]. One hundred and sixty-nine of these 340 articles were rejected for the reasons shown in *Table 1*, leaving 171 articles remaining in the final analysis.

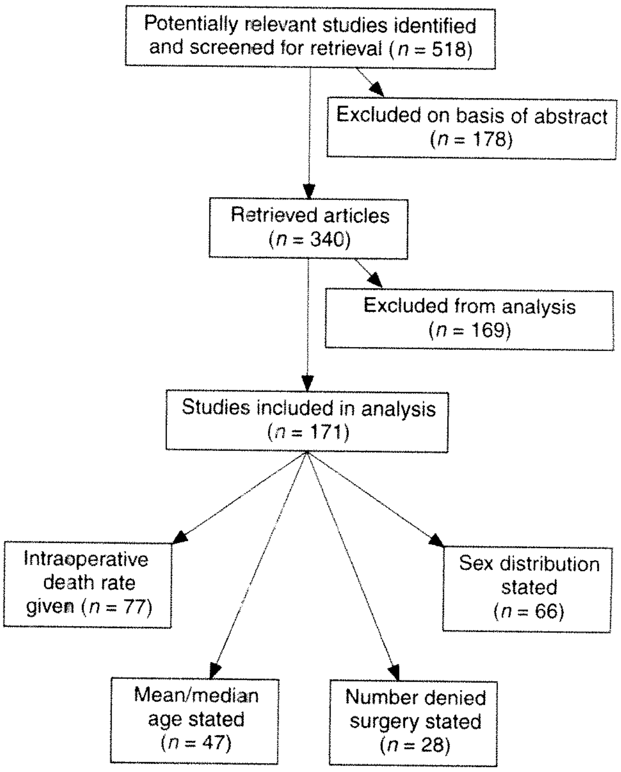
Potentially relevant studies identified and screened for retrieval (n = 518)
Excluded on basis of abstract (n = 178)
Retrieved articles (n = 340)
Excluded from analysis (n = 169)
Studies included in analysis (n = 171)
Intraoperative death rate given (n = 77)
Sex distribution stated (n = 66)
Mean/median age stated (n = 47)
Number denied surgery stated (n = 28)

Fig. 2 Study flow diagram

Table 1 Articles rejected from analysis (n = 169)

Reason for rejection	No.
Selected group of patients (octogenarians)	35 (10)
RAAA not defined explicitly	33
Inadequate data given	16
Not English language	13
Case reports	12
Editorials	10
No RAAA in series	9
Non-ruptured aneurysms included in series	6
Review of surgical methods	5
Other	30
Total	169

References available on application to author. RAAA, ruptured abdominal aortic aneurysm

Study characteristics (*Tables 2, 3 and 4*)

The 171 articles included in the analysis gave details of 21 523 patients over 43 years (mid-timepoints 1954–1997)[8–11,13,22–187]. One hundred and sixty-four articles identified the period of time covered in their study thus allowing the calculation of a mid-timepoint. For the

Table 2 Studies quoting in-hospital mortality in chronological order according to mid-date of study

Reference	Year of publication	*n*	Deaths	Study type[14]	Mid-date of study	Average age (years)	No. of women	No. who refused surgery
Javid *et al.*[22]	1955	4	2 (50)	2b	7 August 1954	62	0 (0)	
Gwathmey *et al.*[23]	1959	16	15 (94)	2b	1 January 1956			
Voorhees *et al.*[24]	1963	34	26 (76)	2b	31 August 1957			
Stallworth *et al.*[25]	1962	23	13 (57)	2b	9 April 1958			
Szilagyi *et al.*[26]	1966	79	46 (58)	2b	31 December 1958			
Hardin[27]	1964	12	8 (67)	2b	31 August 1959			
Lawrence *et al.*[28]	1966	65	34 (52)	2b	30 December 1959			
May *et al.*[29]	1968	41	28 (68)	2b	1 January 1960		5 (12)	
Sanger *et al.*[30]	1964	16	7 (44)	2b	1 June 1960	64	1 (6)	
Tracey and Reeve[31]	1965	27	12 (44)	2b	31 October 1961	70·2	4 (15)	5
van Heeckeren[32]	1970	57	34 (60)	2b	16 August 1962			4
Chiariello *et al.*[33]	1974	87	18 (21)	2b	1 January 1964		6 (7)	
Alpert *et al.*[34]	1970	40	17 (43)	2b	31 December 1964		6 (15)	
Graham *et al.*[35]	1971	90	38 (42)	2b	31 December 1965	72·1	10 (11)	
Taheri[36]	1970	26	10 (38)	2b	6 August 1967	64	3 (12)	
Gardner *et al.*[37]	1978	84	56 (67)	2b	18 June 1968	72·5	7 (8)	
Rantakokko *et al.*[38]	1983	65	41 (63)	2b	31 December 1968			11
Johnston *et al.*[39]	1977	43	32 (74)	2b	1 July 1969			0
Fielding *et al.*[13]	1981	174	74 (43)	2b	31 December 1969			2
Bremner *et al.*[40]	1977	31	18 (58)	2b	30 September 1970			7
Morris *et al.*[41]	1975	44	25 (57)	2b	31 December 1970			6
Nicol[42]	1980	21	11 (52)	2b	1 July 1972			
Hyde *et al.*[43]	1981	64	22 (34)	2b	2 July 1974			
Hepp *et al.*[44]	1981	62	34 (55)	2b	31 December 1974			
Serrano Hernanado *et al.*[45]	1985	23	10 (43)	2b	1 January 1976			
Marsh[46]	1980	29	17 (59)	2b	30 December 1977			6
Donaldson *et al.*[47]	1985	81	35 (43)	2b	31 December 1977	70·5	16 (20)	
Murphy *et al.*[48]	1990	172	85 (49)	2b	31 December 1977	69·8	26 (15)	
Pasch *et al.*[49]	1984	54	38 (70)	2b	1 July 1978			
Jenkins *et al.*[50]	1986	174	57 (33)	2b	2 July 1979		33 (19)	
Doorly *et al.*[51]	1982	13	4 (31)	2b	1 October 1979			
Snider[52]	1987	19	7 (37)	2b	1 July 1980			
Bodily *et al.*[53]	1985	37	23 (62)	2b	1 July 1981	71·6	9 (24)	
Cogbill *et al.*[54]	1988	70	46 (66)	2b	1 July 1981	70	12 (17)	
Gangahar *et al.*[55]	1987	53	22 (42)	2b	2 July 1981			
Campbell *et al.*[56]	1986	52	29 (56)	2b	30 December 1981	75	3 (6)	9
Cohen *et al.*[57]	1991	70	47 (67)	2b	31 December 1981			
Amundsen *et al.*[58*]	1987	103	65 (63)	1b	31 May 1982			0
Rohrer *et al.*[59]	1988	65	24 (37)	2b	1 July 1982	73	11 (17)	0
Martin *et al.*[60]	1988	58	15 (26)	2b	31 December 1982	71	8 (14)	
Mealy and Salman[61]	1988	78	48 (62)	2a	31 December 1982			
Meddings *et al.*[62]	1991	49	23 (47)	2a	1 January 1983			
Samy *et al.*[63]	1994	130	52 (40)	2b	31 December 1984			
Stonebridge *et al.*[64]	1993	227	61 (27)	2b	31 December 1984	71		
Harris *et al.*[65]	1991	113	72 (64)	2b	31 December 1984			0
Gloviczki *et al.*[66]	1992	214	97 (45)	2b	31 December 1984			
McCready *et al.*[67]	1993	206	101 (49)	2b	31 December 1984			
Hannan *et al.*[68]	1992	1659	896 (54)	2b	31 December 1984			
Archie[69]	1992	13	5 (38)	2b	1 April 1986	70·6	3 (23)	
Panneton *et al.*[70*]	1995	112	55 (49)	2b	1 April 1986	72·4	15 (13)	
Halpern *et al.*[71]	1997	96	58 (60)	2b	16 April 1986		19 (20)	
Farooq *et al.*[72]	1996	124	69 (56)	2b	2 July 1986			
Johnston[73]	1994	147	73 (50)	1b	16 August 1986			
Collin *et al.*[74*]	1989	75	27 (36)	1b	31 October 1986		10 (13)	11
van Dongen *et al.*[75]	1998	309	78 (25)	2b	31 December 1986	71	35 (11)	
Sayers *et al.*[76]	1997	278	148 (53)	2b	2 July 1987		41 (15)	
Barry *et al.*[77]	1998	239	102 (43)	2b	1 January 1988			
Meesters *et al.*[78]	1994	113	49 (43)	2b	1 July 1988			
Magee *et al.*[79]	1992	77	27 (35)	2b	31 December 1988			
Evans *et al.*[80]	2000	586	199 (34)	1b	1 July 1989		105 (18)	

Table 2 Continued

Reference	Year of publication	*n*	Deaths	Study type[14]	Mid-date of study	Average age (years)	No. of women	No. who refused surgery
Seiwert *et al.*[81]	1995	119	54 (45)	2b	30 September 1989		12 (10)	
Darling *et al.*[82]	1996	104	29 (28)	2b	31 December 1989	72	17 (16)	0
Martinez *et al.*[83]	1997	84	48 (57)	2b	31 December 1989	74	9 (11)	
Anastassov[84]	1993	11	1 (9)	2b	2 July 1990			
Akkersdijk *et al.*[85]	1994	709	309 (44)	2b	2 July 1990			
Wen *et al.*[86]	1996	1203	482 (40)	2b	2 July 1990			
Choksy *et al.*[87]	1999	59	30 (51)	2a	31 December 1990			
Ho *et al.*[88]	1999	41	20 (49)	2b	2 July 1991			
Piotrowski *et al.*[89]	1995	80	45 (56)	2b	30 June 1992	71·9	14 (18)	
Miani *et al.*[90]	1998	152	37 (24)	2b	1 July 1992			
Dardik *et al.*[91]	1998	527	250 (47)	2a	31 December 1992	71·9	108 (20)	
Kazmers *et al.*[92]	1998	427	202 (47)	2b	31 December 1993			
Sebesta *et al.*[93]*	1998	103	63 (61)	2b	31 December 1993			
Lobo *et al.*[94]	1999	68	28 (41)	2b	16 July 1995			

Values in parentheses are percentages. *Also gave 30-day mortality (*Table 3*)

remaining seven articles[11,121,136,154,155,164,186] the publication date was used in the analysis. Seventy studies quoted in-hospital mortality[13,22–57,59–69,71–73,75–92,94], 37 quoted 30-day mortality[9–11,95–128], four quoted both figures[58,70,74,93], and 60 failed to define their criteria for operative mortality[8,129–187]. Seventy-seven studies gave details of intraoperative deaths, 66 reported gender ratio, 47 stated the mean or median age of patients undergoing surgery and 28 stated the number of patients refused surgery. The majority of studies (149) were Blankensteijn level 2b (retrospective hospital-based studies), ten were level 2a (prospective hospital-based studies) and 12 were level 1b (retrospective community-based studies). The details of each study are shown in *Tables 2*, *3* and *4*.

Pooled mortality

The overall pooled estimate of operative mortality rate for all 171 studies in the analysis was 48 (95 per cent confidence interval (c.i.) 46 to 50) per cent. There was considerable heterogeneity between study results on the log odds scale ($Q = 996{\cdot}4$ on χ^2_{170} ($P < 0{\cdot}001$), between-study variance = 0·17 (method of moments)). The method of classifying operative mortality had little effect on the estimated mortality rate (*Table 5*) and heterogeneity was present within each classification ($P < 0{\cdot}001$ in all cases). The intraoperative mortality rate, estimated from the 77 studies that reported it, was 15 (95 per cent c.i. 13 to 17) per cent; again heterogeneity was present ($Q = 330{\cdot}4$ on χ^2_{76} ($P < 0{\cdot}001$), between-study variance = 0·32). Using these 77 studies, the postoperative mortality rate (the percentage of patients who survive surgery but subsequently die) was estimated to be 40 (95 per cent c.i. 37 to 43) per cent (*Table 5*) ($Q = 292{\cdot}1$ on χ^2_{76} ($P < 0{\cdot}001$), between-study variance = 0·18). In the 66 studies that reported the gender ratio of their study population, the mean percentage of females undergoing surgery was 13 (range 0–30) per cent.

Meta-regression analysis

Including mid-date of study as a co-variate in a meta-regression model suggests an approximately linear constant reduction in operative mortality over the 50 years of practice covered by the literature (171 studies, $P = 0{\cdot}001$) (*Fig. 3*). The regression equation (s.e. in parentheses) is $\ln(\text{odds death}) = 27{\cdot}44(8{\cdot}19) - 0{\cdot}0139(0{\cdot}0041) \times$ mid-date. When the regression line is transformed on to the proportion scale (*Fig. 3*), this translates into a 3·5 per cent reduction in mortality per 10 years over the analysis period. After accounting for this source of between-study heterogeneity, residual heterogeneity remained with the between-study variance being estimated as 0·21 (restricted maximum likelihood estimate, value for method of moments is 0·15).

To determine whether the observed improvement in operative mortality rate is due to changes in intraoperative or postoperative factors, meta-regression analyses of intraoperative and postoperative mortality including mid-date of study as a co-variate were performed using the 77 papers that quoted intraoperative death rates. Little evidence of an association between intraoperative mortality and mid-date was observed (slope coefficient(s.e.) = 0·005(0·008), $P = 0{\cdot}53$), suggesting that it has remained approximately constant over the period of the analysis (*Fig. 4*). Although this suggests that the improvements in overall mortality are likely to be due to improvements in postoperative care rather than operative technique, meta-regression analysis of

Table 3 Studies quoting 30-day mortality in chronological order according to mid-date of study

Reference	Year of publication	*n*	Deaths	Study type[14]	Mid-date of study	Average age (years)	No. of women	No. who refused surgery
Kouchoukos *et al.*[95]	1967	36	22 (61)	2b	1 April 1962		2 (6)	
Yashar *et al.*[96]	1972	32	18 (56)	2b	30 October 1964			
Johnson *et al.*[97]	1980	69	46 (67)	2b	31 December 1964			
Bosman and Stubbe[10]	1981	209	88 (42)	2b	30 September 1968		17 (8)	0
Salerno *et al.*[98]	1981	17	13 (76)	2b	31 December 1969			
David and Bernatz[11]	1971	94	38 (40)	2b	1 May 1971			
Hertzer[99]	1980	43	20 (47)	2b	2 July 1971			
Soreide *et al.*[100]	1982	125	73 (58)	2b	1 July 1973	70		
Pilcher *et al.*[101]	1980	87	48 (55)	2b	30 September 1973			
Morishita *et al.*[102]	1986	20	9 (45)	2b	16 October 1976			
Makin[103]	1983	68	34 (50)	2b	31 December 1976			
Lawler[104]	1984	43	17 (40)	2b	30 April 1977	70	7 (16)	
Hoffman *et al.*[105]	1982	152	58 (38)	2b	1 July 1977		17 (11)	
Bengtsson and Bergqvist[106]	1993	61	35 (57)	2a	31 December 1978	73	8 (13)	
Johansson and Swedenborg[107]	1994	51	30 (59)	2a	2 July 1989			
Poulias *et al.*[108]	1994	80	33 (41)	2b	30 March 1980			
Jaakkola *et al.*[109]	1996	48	31 (65)	2b	31 December 1980	67	4 (8)	
Soisalon-Soininen *et al.*[110]	1995	364	178 (49)	2b	1 April 1981		55 (15)	
Amundsen *et al.*[58]*	1987	103	62 (60)	1b	31 May 1982			
Johansen *et al.*[111]	1991	180	124 (69)	2b	31 December 1984			1
Burke and Sannella[112]	1993	117	61 (52)	2b	31 December 1984			
Bauer *et al.*[113]	1993	314	92 (29)	2b	29 January 1985	69·6	24 (8)	
AbuRahma *et al.*[114]	1991	73	45 (62)	2b	1 July 1985	72	8 (11)	
Panneton *et al.*[70]*	1995	112	51 (46)	2b	1 April 1986	72·4	15 (13)	
Collin *et al.*[74]*	1989	75	27 (36)	1b	31 October 1986		10 (13)	
Sasaki *et al.*[115]	1997	27	6 (22)	2b	15 February 1988			
Chen *et al.*[116]	1996	157	73 (46)	2b	31 December 1988	74	36 (23)	
Lindholt *et al.*[117]	1995	22	8 (36)	2a	31 December 1988			
Marty-Ane *et al.*[118]	1995	61	26 (43)	2b	31 December 1989	72·1	6 (10)	
Norman *et al.*[119]	1998	283	102 (36)	2b	31 December 1989		41 (14)	
Wilson *et al.*[120]	1993	9	2 (22)	2b	5 May 1990			
Walsh *et al.*[121]	1992	47	24 (51)	2b	20 January 1992			11
Gordon *et al.*[122]	1994	91	35 (38)	2b	6 April 1992			
Kantonen *et al.*[123]	1999	454	209 (46)	2a	31 December 1992			
Adam *et al.*[9]	1998	377	148 (39)	1b	31 December 1992			
Ascher *et al.*[24]	1999	10	1 (10)	2b	1 April 1993	73·4	3 (30)	
Lazarides *et al.*[125]	1997	40	22 (55)	1b	30 October 1993	72·2	3 (8)	
Sebesta *et al.*[93]*	1998	103	58 (56)	2b	31 December 1993			
Maziak *et al.*[126]	1998	88	35 (40)	2b	30 November 1994	72·5	19 (22)	
Prance *et al.*[127]	1999	79	38 (48)	2b	2 July 1995			
Basnyat *et al.*[128]	1999	133	85 (64)	1b	2 March 1997			

Values in parentheses are percentages. *Also gave in-hospital mortality (see *Table 2*)

postoperative mortality similarly did not demonstrate any significant change over time either (slope coefficient(s.e.) = – 0·004(0·006), P = 0·50). In fact, taken in isolation, these 77 papers showed little evidence of a relationship between overall operative mortality and mid-date of study (slope coefficent(s.e.) = – 0·002(0·007), P = 0·73). However, the estimated operative mortality from these studies is 49 (95 per cent c.i. 46 to 50) per cent, which is very similar to that estimated from the overall analysis. The lack of an association between this subgroup of studies and mid-date of study could be explained by the reduced power of the analysis compared with that using all studies[188], or may be due to the studies representing a selectively biased subgroup, for which there is good evidence (see 'Assessment of publication bias' below).

Average age (47 studies) was not associated with overall operative mortality (slope(s.e.) = – 0·019(0·033), P = 0·56), and neither was the proportion of women (66 studies) (slope(s.e.) = – 0·577(1·303), P = 0·66), but there was a positive association between intraoperative death rates and the proportion of women (32 studies, slope(s.e.) = 4·450(1·940), P = 0·02). Intraoperative death rates were not associated with age (25 studies, slope(s.e.) = 0·045(0·051), P = 0·38).

Table 4 Studies that did not define mortality in chronological order according to mid-date of study

Reference	Year of publication	*n*	Deaths	Study type[14]	Mid-date of study	Average age (years)	No. of women	No. who refused surgery
Couch *et al.*[129]	1970	29	18 (62)	2b	16 April 1961			
Mannick *et al.*[130]	1964	25	8 (32)	2b	30 September 1961	65		
Shumacker *et al.*[131]	1973	158	87 (55)	2b	1 January 1963			0
Louw *et al.*[132]	1972	66	46 (70)	2b	28 September 1963			0
Bucknam[133]	1972	74	35 (47)	2b	1 July 1964			
DiGiovanni *et al.*[134]	1975	107	73 (68)	2b	1 July 1964			6
Dent[135]	1980	120	70 (58)	2b	2 July 1967			
Magee *et al.*[136]	1997	35	13 (37)	1b	3 October 1967	68		5
Rainer *et al.*[137]	1973	19	7 (37)	2b	29 February 1968			
Crawford *et al.*[138]	1981	60	14 (23)	2b	1 March 1968			
Byrne and Rayhanabad[139]	1977	31	22 (71)	2b	14 November 1969			
Appleberg *et al.*[140]	1980	134	69 (51)	2b	31 December 1969			
Hicks *et al.*[141]	1975	56	31 (55)	2b	17 March 1970	69	9 (16)	
Sink *et al.*[142]	1976	33	20 (61)	2b	2 July 1970		6 (18)	
Mulherin and Edwards[143]	1980	57	23 (40)	2b	2 July 1970			
Schmidt[144]	1973	6	0 (0)	2b	15 November 1970		0 (0)	
Lawrie *et al.*[145]	1979	61	9 (15)	2b	2 July 1971	77·5	4 (7)	
Wakefield *et al.*[146]	1982	116	60 (52)	2b	31 December 1971	68·7	11 (9)	
Young *et al.*[147]	1977	21	12 (57)	2b	30 September 1972			
Shannon[148]	1979	36	10 (28)	2b	31 December 1972			
Butler *et al.*[149]	1978	76	44 (58)	2b	30 December 1973		2 (3)	3
Lassonde *et al.*[150]	1981	36	15 (42)	2b	2 July 1974	67	4 (11)	
Ruberti *et al.*[151]	1985	238	130 (55)	2b	2 July 1974			0
Armour[152]	1977	5	1 (20)	2b	30 November 1974			3
McCabe *et al.*[153]	1981	73	38 (52)	2b	30 December 1974	73	12 (16)	
Nash[154]	1975	13	3 (23)	2b	19 July 1975			2
Moore[155]	1976	18	8 (44)	2b	1 January 1976	73	2 (11)	
Lundell and Norback[156]	1983	185	161 (87)	2b	1 January 1976			
Botta *et al.*[157]	1983	16	8 (50)	2b	2 July 1977			
Bandyk *et al.*[158]	1981	50	33 (66)	2b	31 December 1977	70·8	7 (14)	
Snellen *et al.*[159]	1984	93	35 (38)	2b	31 December 1977			
Munoz *et al.*[160]	1988	46	32 (70)	2b	1 January 1980	70·5		
Johansson and Swedenborg[161]	1986	8	3 (38)	2a	1 July 1980			
Romeiser *et al.*[162]	1988	28	11 (39)	2b	1 July 1980			
Payne *et al.*[163]	1985	13	4 (31)	2b	31 December 1980			
Harbrecht *et al.*[164]	1982	8	6 (75)	2b	22 February 1981		0 (0)	
Gregory *et al.*[165]	1990	71	36 (51)	2b	1 July 1981			
Vohra *et al.*[166]	1988	92	36 (39)	2b	2 July 1983			
Thomas and Stewart[167]	1988	75	41 (55)	2b	1 January 1984			20
Olsen *et al.*[168]	1991	218	81 (37)	2b	1 January 1984			
Budd *et al.*[169]	1989	88	60 (68)	2a	31 December 1984			27
Katz *et al.*[170]	1994	1829	911 (50)	2b	1 July 1985			
Scott *et al.*[171]	1992	63	19 (30)	1b	31 December 1985		8 (13)	
Bradbury *et al.*[172]	1998	673	249 (37)	1b	2 July 1986		119 (18)	
Chang *et al.*[173]	1988	4	3 (75)	2b	29 September 1986			
Hajarizadeh *et al.*[174]	1995	105	42 (40)	2b	1 January 1988			
Joseph *et al.*[175]	1989	14	4 (29)	2b	28 March 1988			
Katz and Kohl[176]	1994	99	57 (58)	2b	1 July 1988	73·4	14 (14)	6
Satta *et al.*[177]	1997	51	24 (47)	2b	31 December 1988			
Szostek *et al.*[178]	1993	211	127 (60)	2b	1 July 1989		12 (6)	
Hardman *et al.*[179]	1996	154	60 (39)	2b	2 July 1989	71·4	24 (16)	17
Koskas and Kieffer[180]	1997	158	73 (46)	1b	2 July 1989	73·1	16 (10)	
Cloutier *et al.*[181]	1996	106	43 (41)	2b	15 November 1990	71·2	16 (15)	
Bradbury *et al.*[182]	1995	60	25 (42)	2b	1 April 1991			5
Morehouse *et al.*[183]	1995	84	35 (42)	2b	2 July 1991		10 (12)	
Subramaniam *et al.*[184]	1998	18	12 (67)	2b	31 March 1992			
Paaske *et al.*[185]	1994	36	8 (22)	1b	30 August 1992			
Podlaha *et al.*[8]	2000	92	44 (48)	2b	1 June 1994	71	19 (21)	
Browning *et al.*[186]	1995	49	22 (45)	2b	1 March 1995		6 (12)	
Burger *et al.*[187]	1999	52	19 (37)	2b	16 August 1995		5 (10)	

Table 5 Operative mortality rate estimates by method of classification and intraoperative and postoperative mortality rate estimates

Mortality outcome	No. of studies	Mortality rate
Overall	171	48 (46, 50)
In-hospital	74	48 (45, 51)
30 day	41	48 (45, 52)
Not stated	60	48 (45, 52)
Intraoperative	77	15 (13, 17)
Postoperative	77	40 (37, 43)

Values in parentheses are 95 per cent confidence intervals

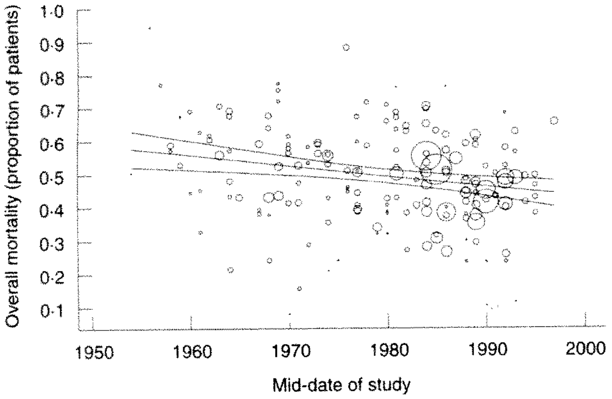

Fig. 3 Meta-regression plot of mid-date of study against operative mortality. Outer lines are 95 per cent confidence intervals (171 studies, $P = 0.001$ for gradient of zero). Circles represent individual studies; the size of the circle is proportional to the inverse of the variance of the mortality estimate for that study, indicating the relative influence in the meta-analysis

The 41 studies that reported both average age and proportion of women demonstrated a significant relationship between these two factors; studies with a high proportion of women had a higher average age ($P = 0.02$ using standard linear regression). The number of patients turned down for surgery (expressed as a percentage) was not associated with operative mortality (28 studies, $P = 0.51$) or intraoperative mortality (12 studies, $P = 0.68$). The 77 studies that quoted the average age of patients undergoing RAAA repair suggest that this has increased over time (*Fig. 5*). A quadratic curve fitted the relationship well, indicating that average age has reached a plateau in the past decade.

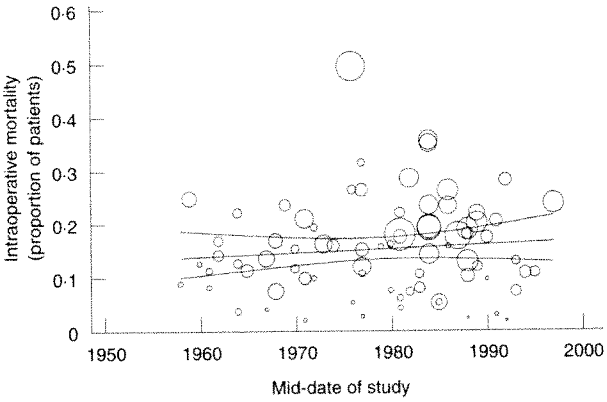

Fig. 4 Meta-regression plot of mid-date of study against intraoperative mortality. Outer lines are 95 per cent confidence intervals (77 studies, $P = 0.53$ for gradient of zero). Circles represent individual studies; the size of the circle is proportional to the inverse of the variance of the mortality estimate for that study, indicating the relative influence in the meta-analysis

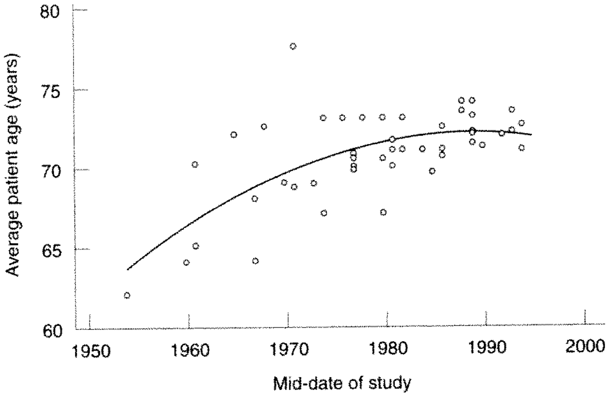

Fig. 5 Scatter plot of mid-date of study against age (47 studies, $R^2 = 0.40$, $P = 0.02$)

Assessment of study quality

Thirty-nine studies were multicentre and the remaining 132 were conducted in a single centre. Little difference was observed between the overall operative mortality rates for these types of study (48 (95 per cent c.i. 45 to 50) per cent for the single-centre *versus* 49 (95 per cent c.i. 44 to 55) per cent for the multicentre studies; $P = 0.52$). Five studies used coded computerized records. Although this was a small proportion of the total number of studies, there was some evidence that mortality rates were lower in the coded records group (45 (95 per cent c.i. 35 to 56) *versus* 48 (95 per cent c.i. 46 to 50) per cent; $P = 0.10$). The subgroup of 149 studies that were categorized as Blankensteijn level 2b had a pooled mortality rate of 48 (95 per cent c.i. 38 to 59) per cent compared with 52 (95 per cent c.i. 39 to 65) per cent for the ten level 2a studies and 43 (95 per cent c.i. 36 to 50) per cent for the 12 level 1b studies. Hence, there would appear to be

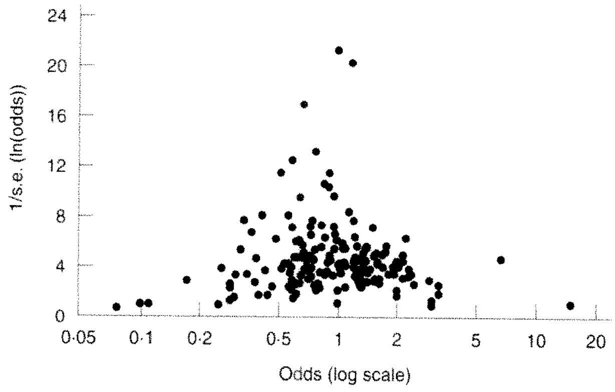

Fig. 6 Funnel plot of operative mortality for all 171 studies included in analysis

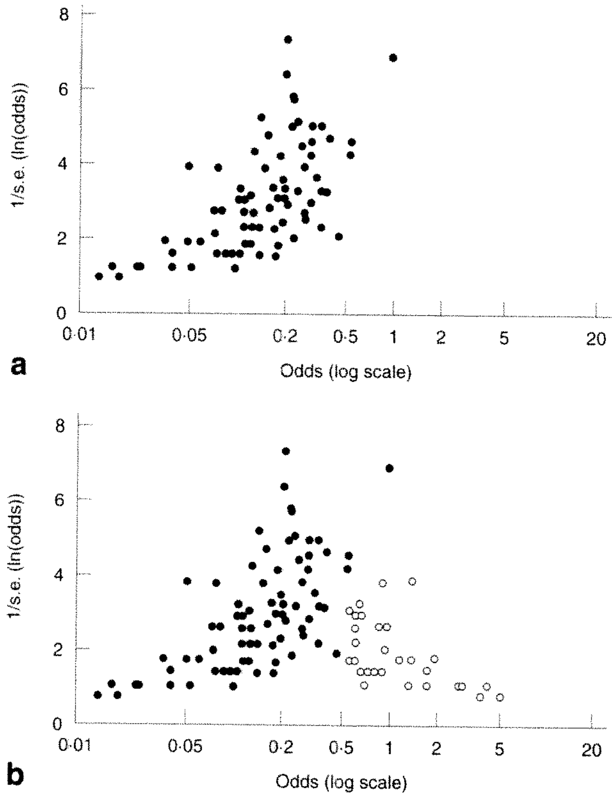

Fig. 7 Funnel plots for intraoperative mortality showing **a** studies in the analysis (filled circles, *n* = 77) and **b** with the addition of those predicted to be missing by trim and fill method (open circles, *n* = 29)

potentially some differences in estimates for overall mortality for different subgroups, but the very uneven spread of studies across these subgroups with one category always dominating makes interpretation difficult.

Assessment of publication bias

A funnel plot for all 171 studies included in the analysis examining overall operative mortality was constructed in order to assess the degree of publication bias (*Fig. 6*). Briefly, any asymmetry around a vertical axis may indicate the presence of such bias[189]. This plot was highly symmetrical, indicating that publication bias is unlikely to have an important influence on the overall analysis of operative mortality. However, when a similar plot was constructed for intraoperative mortality (*n* = 77) (*Fig. 7a*), extreme asymmetry was evident indicating possible bias. The suspected 'missing' studies, in the bottom right hand corner of the plot, correspond to those that would report a high intraoperative mortality rate.

Using the method of trim and fill, prediction of potentially missing studies was attempted (adopting a fixed-effect model and using the estimator previously described as L_0[17,18] for the missing studies). For the overall analysis of operative mortality this predicted no missing studies, but for intraoperative mortality 29 missing studies were predicted (*Fig. 7b*). If those studies that are potentially missing are imputed into the analysis, the result for the random-effect pooled estimate of intraoperative mortality rate changes from 15 (95 per cent c.i. 13 to 17) to 20 (95 per cent c.i. 18 to 23) per cent.

For completeness, funnel plots adjusted by the co-variate midpoint of study (by plotting the residuals from the corresponding meta-regression models) to remove any distortion caused by this known outcome modifier were examined. The visual impact of these funnel plots, and the results of trim and fill, were virtually identical to the unadjusted funnels, indicating that the effect of time was not an explanation for funnel appearance (plots and analyses not shown). Additionally, funnel plots were constructed for overall mortality for those studies that did, and did not, report intraoperative mortality. These funnels were reasonably symmetrical (plots not shown).

Discussion

Results

This study demonstrates that, since RAAA repair was first described 50 years ago, the operative mortality reported in the published literature is approximately 48 per cent. This is the overall figure for all reports from this entire time period. As this figure has shown a gradual reduction over time, operative mortality at the start of the analysis period was higher than 48 per cent, and is now lower. Using the regression line shown in *Fig. 3* the operative mortality rate estimate for 1960 is 55 per cent, for 1980 is 48 per cent and for 2000 is 41 per cent. This improvement over time is not likely to continue at the same rate since this would imply that eventually the operative mortality of RAAA repair would become zero. It is more likely, if future figures were to be plotted on the same axes, that the slope of the regression line would become less steep, tending eventually to a lower limit. The timescale over which this may occur, or the lower limit to which operative mortality will reach, cannot be estimated from this study. If it were possible to identify the causes of this gradual improvement over time it may be possible to accelerate this improvement by targeting these factors.

Progressively increasing numbers of women and elderly patients have undergone RAAA repair over the analysis period. No association was found between the operative mortality of RAAA repair and average age, gender or the number of patients turned down for surgery by the operating surgeon. However, because of missing data, the numbers of studies contributing to the meta-regression analyses was often greatly reduced, and inadequate statistical power cannot be ruled out as the explanation for the lack of associations found[188]. Female sex, but not increasing age, was associated with an increased *intraoperative* mortality rate. Despite the increase in numbers of women undergoing surgery, overall intraoperative death rates did not change over time. These conclusions relating to intraoperative mortality should be viewed with caution, however, because of the potentially biased sample of data it is based on, as suggested by the publication bias assessment.

Patients who survive RAAA repair often have a prolonged hospital stay and are resident for long periods on critical care wards at considerable financial cost. Given the persistent high mortality rate demonstrated, this may lead some readers to the conclusion that the cost in relation to benefit of RAAA repair is too high to justify continuing to operate on these patients. However, this study also demonstrates that currently about 59 per cent of patients will survive and, since these patients have a long-term survival similar to that of the general population[190], surgery seems justified. Also, a recent study by Patel *et al.*[191] demonstrated that the cost per quality-adjusted life year (QALY) gained was US $10 754 (approximately £7347, October 2001) which was similar to that for a three-vessel coronary artery bypass graft (US $9500 (approximately £6490) per QALY gained), and that RAAA repair is cost-effective if the operative mortality rate is less than 88 per cent.

There was a large degree of heterogeneity (which necessitated use of a random-effects model) among the studies included in the analysis, with operative mortality varying from as low as 0 per cent[144] to as high as 94 per cent[23]. Some studies with more extreme mortality estimates had very small numbers but others had relatively large numbers[75,90,101]. One study in particular was identified as having particularly extreme values despite containing a large number of patients[156]. This can be seen as the high outlier in the middle of *Figs 3* and *5*, and also as the top right outlier in *Fig.* 7. Re-examining this paper, the data presented could be interpreted in ways other than that used in the analysis. However, since excluding this study and repeating the analysis caused very little change to the results compared with those presented above, it has remained in the analysis. It may represent an extreme of the heterogeneity seen in the analysis as a whole.

The reasons for the heterogeneity are not clear. Many factors unique to one particular centre may affect the results from RAAA repair in that centre. For example, local differences in the socioeconomic status of the patient population, healthcare resources and vascular surgical subspecialization may all affect outcome, and so different centres are likely to have different figures. Therefore, while the figures derived from the present study are a useful guide, local audit should be maintained to determine local estimates.

Potential biases

While every effort was made to ensure that all literature on RAAA was reviewed for possible inclusion in the analysis, it is possible that some eligible studies may have been missed on either electronic or manual literature searches. While the inclusion of any potentially missed studies is desirable, probably much more serious is the suspected selective outcome reporting suggested by the publication bias assessment[192]. The fact that little bias was evident in the overall analysis but considerable bias was present when examining a subgroup suggests that, while most authors report general figures accurately, if detailed figures do not reflect well upon themselves (high intraoperative mortality rate), they may be tempted not to include these in their report.

Further data obtained from the sensitivity analysis support this. There was no difference between the multicentre and single-centre studies in the values for operative mortality, but the single-centre studies had a lower estimate for intraoperative mortality, similar to that obtained from the overall analysis. The estimate for intraoperative mortality for the multicentre trials was similar to that obtained using a trimmed and filled estimate after the identification of potential bias in the group of studies reporting intraoperative mortality. It may be that single centres with high intraoperative mortality rates are selectively not reporting these when reporting their figures for operative mortality. It is possible, since the figures from individual centres are usually anonymized when part of a multicentre study, that there is no potential for any particular institution to be discredited and so mortality rates are more likely to be reported accurately.

Inferences

It is not possible to explain whether the temporal change in operative mortality seen in this study is due to postoperative or intraoperative factors. The potential publication bias in the subgroup of studies reporting intraoperative mortality severely limits the strengths of any conclusions drawn from these data.

The failure significantly to improve operative mortality over time may be due to increasing numbers of elderly patients being admitted to surgery. The lack of association between increasing age and mortality is unexpected. Reports focusing on elderly patients have reported the operative mortality rate in this group as 58–68 per cent[193–195], significantly higher than the operative mortality estimate in the present study. The average ages in this analysis are much less than 80 years, and it may be that elderly patients are being routinely excluded from surgery unless part of a clinical study examining this particular patient group. While the inclusion of studies that examined elderly patients may have increased the ability of this study to identify the expected association between age and mortality, it potentially would have altered the estimate for the primary outcome measure of the study, the operative mortality of RAAA repair.

Further research

The large volume of literature on the subject of RAAA would appear to make it an ideal subject for meta-analytical techniques. Many studies of RAAA focus on predictors of mortality[7,140], differences between dedicated vascular and 'general' surgeons[50], and operative techniques[106,118]. During the conduct of this study data were extracted from each paper that identified predictors of mortality in its patient group. Also, factors such as specialization of the surgeon and whether surgery was performed in secondary or tertiary referral centres were recorded where available. While it was hoped that these data might elucidate the factors responsible for high mortality rates, the small numbers of articles giving such data and the variability in reporting of the data meant that any analysis would have been meaningless. The institution of prospective national audit, such as that of the Vascular Surgical Society of Great Britain and Ireland, is likely to prove more useful in this respect.

Issues arising in the process of carrying out this meta-analysis lead the authors to make some recommendations for the future reporting of outcomes in surgical research. Sixty articles did not give a definition of mortality and 30 articles were not included in the analysis since they did not give an explicit definition for RAAA. If reporting had been slightly more detailed on these two accounts more studies may have been included in the meta-analysis and more precise results obtained. The funnel plot for the assessment of publication bias in studies reporting intraoperative deaths (*Fig. 7a*) suggests that many papers with high intraoperative mortality are either not published or the figures themselves are not reported. It is more difficult to recommend a workable solution to this problem, especially as measures taken to alleviate publication bias in the randomized trial literature, such as prestudy registration, are not feasible in this or other contexts where the evidence is primarily observational.

The amount of repeated publication of data was surprising. The inclusion of covertly duplicated publications has been shown to affect the results of meta-analyses[196]. While comparison of figures from the same institution for different time periods is often useful to allow inferences regarding the effect of policy changes to be made, a more open acknowledgement of whether these data have been published previously is recommended to avoid allegations of covert dual publication.

Conclusion

This study demonstrates that RAAA is one of the most significant challenges facing healthcare services today; it is likely to become even more important in the future. The authors have highlighted the high operative mortality rate among patients who reach hospital alive and, in contrast to other recent studies[197,198], have demonstrated a limited, but definite, improvement in operative mortality over time. The effects of changes in the demographic features of patients undergoing surgery on operative mortality rates are not clear from this study. It makes clinical sense that older patients have a higher operative mortality rate than younger patients and, while no statistically significant relationship between age and operative mortality was demonstrated, this does not mean that an association does not exist. It is entirely plausible that the increase in the average age of patients over time may explain the limited reduction in mortality observed.

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Vascular Surgical Society

Economic evaluation of a randomized clinical trial of haemodilution with cell salvage in aortic surgery

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Background: This study evaluated the costs of acute normovolaemic haemodilution (ANH) and intraoperative cell salvage (ICS) *versus* homologous blood transfusion in aortic surgery in a prospective multicentre randomized trial.

Methods: One hundred and forty-five patients were randomized either to standard transfusion practice (homologous) or to a combination of ANH and ICS (autologous). Costs for each inpatient admission were identified. Cell salvage costs were assigned on the assumption that 50 operations were done each year employing a trained cell salvage operator. The results were analysed statistically using bias-corrected bootstrap analysis.

Results: Patients who had transfusion of homologous blood received some 251 units and those having a homologous transfusion received 103 units ($P = 0.008$). There was no difference in morbidity, mortality and duration of hospital stay. Transfusion-related mean costs were similar at £340 for patients having a homologous transfusion and £357 for those receiving autologous blood (mean difference £17 (95 per cent confidence interval (c.i.) −£184 to £174); *P* not significant). There was also no significant difference in mean overall costs: £5859 for homologous and £5384 for autologous transfusion (mean difference −£475 (95 per cent c.i. −£2231 to £1342)). Sensitivity analysis showed that costs remained similar for 20 and 150 operations per annum. Exclusion of a dedicated cell salvage operator reduced autologous transfusion costs but did not have a significant impact on overall cost.

Conclusion: Autologous transfusion is cost neutral in aortic surgery even when surgical activity is low.

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Introduction

Autologous blood transfusion reduces homologous blood requirements in elective aortic surgery[1–4], but the cost effectiveness of this approach remains unproven[5–7]. The NHS Executive in the UK has recommended the implementation of autologous blood transfusion, by cell salvage in particular[8], but surgeons are reluctant to change transfusion practice without conclusive evidence[9]. In a recent systematic review, the majority of economic evaluations of intraoperative cell salvage (ICS) were of poor quality and frequently failed to account for the staff costs and maintenance of cell salvage devices[10].

Autologous Transfusion In Surgery (ATIS) was a randomized trial that addressed the role of autologous transfusion – a combination of acute normovolaemic haemodilution (ANH) and ICS – in elective aortic surgery[3,4,11]. This paper compares the cost of autologous transfusion in aortic surgery to standard (homologous) transfusion practice.

Patients and methods

Following ethics committee approval and fully informed consent in writing, sequential patients undergoing elective infrarenal aortic surgery in eight hospitals in the north-west of England were recruited. Inclusion and exclusion criteria are listed in *Table 1*. Randomization to homologous or autologous transfusion was stratified by computerized minimization for hospital, occlusive or aneurysmal aortic disease, antiplatelet or anticoagulant drugs, and estimated blood volume. This stratification allowed a balanced distribution of factors that could influence transfusion requirements.

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Elective infrarenal aortic reconstruction	Myocardial infarction in previous 6 months
Age above 30 and less than 85 years	Severe angina (American Heart Society)
Haemoglobin concentration > 11 g/dl	Myocardial ischaemia on resting electrocardiography
Platelet count > 150 000 per litre	Aortic stenosis
Written informed consent	Left ventricular ejection fraction < 40 per cent
	Preoperative creatinine level > 200 mmol/l
	Aspartate aminotransferase level > 100 units/l
	Patient refusal of homologous blood
	Haematological disorders excluding either transfusion technique
	Severe pulmonary disease ($P\text{aO}_2$ < 9 kPa on air; FEV_1 < 50 per cent predicted)

$P\text{aO}_2$, arterial partial pressure of oxygen; FEV_1, forced expiratory volume in 1 s

Autologous transfusion

All patients receiving autologous transfusion underwent ANH after induction of anaesthesia to a target haemoglobin concentration of 11 g/dl, as described previously[4,11].

Blood lost during the procedure was salvaged using one of three centrifugal cell salvage devices: Haemonetics® Cell Saver® 5 (Haemonetics, Braintree, Massachusetts, USA), BRAT® 2 (Cobe® Cardiovascular, Arvada, Colorado, USA) or Fresenius CATS (Fresenius Kabi, Bad Homburg, Germany), with comparable efficacy in red cell recovery under standard conditions[12,13].

Transfusion triggers

Whenever salvaged red cells or whole blood units from haemodilution were available, they were given first in patients receiving autologous transfusion. Under all other circumstances, homologous blood was administered when the haemoglobin concentration fell below 8 g/dl or when ischaemic electrocardiographic changes (2-mV ST segment elevation or depression on three-lead monitoring or 12-lead trace) persisted after correction of hypovolaemia.

Data collection and analysis

Data were collected prospectively from admission to discharge. The study adopted an NHS viewpoint, accounting only for costs and benefits accrued or incurred by the hospital (costs and benefits in primary care were not considered). All costs for each hospital episode were identified and enumerated for the financial year 1999–2000 using an ingredients-based approach broken down by staff, capital and consumables. These included ward stay, drugs, theatre time and consumables, diagnostic tests, homologous blood and cross-matching, transfusion consumables, cell salvage machine costs and maintenance contracts. As variables related to costs are seldom normally distributed, the mean should not, as a rule, be used as a descriptive statistic and non-parametric tests would be appropriate. From a financial health planning standpoint, however, median costs are relatively meaningless. Therefore, a bias-corrected bootstrap analysis was used, a validated method for calculating confidence intervals (c.i.) of the mean on these data[14–16].

Data were expressed as means, mean differences and 95 per cent c.i. To ensure generalizability of the results, sensitivity analyses were conducted to evaluate the impact of different levels of activity and different cell salvage devices. It was assumed that small vascular units would perform approximately 20 aortic reconstructions per annum, average units would perform approximately 50 operations per annum, and large units approximately 150 per annum. Continuous variables not related to cost were expressed as medians and ranges or interquartile ranges (i.q.r.), and compared with the Mann–Whitney *U* test.

Theatre costs

A common theatre cost was not assumed, as the transfusion method (in particular ANH) may influence theatre times. Theatre costs were calculated using the duration for each procedure, and including each member of staff present. An overhead was then apportioned by staff grade and time. Staff costs were obtained from Netten and Curtis[17]. The costs of consumables were obtained from regional supplies catalogues.

Transfusion-related costs

The cost of autologous transfusion included ANH consumables and the purchase and operation of a cell salvage device (consumables, staff, maintenance and capital cost). The cost of blood bags for ANH was obtained from Baxter

Healthcare (Newbury, UK). The cost of cell salvage devices was obtained from suppliers' list prices and converted into a net present value based on an annuity factor of 6 per cent (UK Treasury guidance) and a capital lifespan of 8 years. Maintenance costs were based on service agreements stipulated by South Manchester University Hospital (SMUH) with the relevant contractors. Cell salvage consumable costs were obtained from the manufacturers' list prices (*Table 2*). For each patient, cell salvage costs specific to the device used for that individual were assigned on the assumption of an activity of 50 operations per annum with the provision of a cell salvage nurse (midpoint F grade) who was charged at *pro rata* costs based on the time it took to set up, complete each procedure and clear up afterwards. It was assumed that any personnel involved in the treatment of a given patient (including the cell salvage operator) would proceed with other duties once the operation was over.

The cost of homologous blood (£78·88 per unit) was obtained form the Manchester Blood Transfusion Service. All blood-giving sets were accounted for and cross-match charges (£17·92 per test) were obtained from the SMUH pathology department.

Ward stay

Ward and intensive care costs were supplied by the finance departments of each participating hospital. These were attributed by ward type to reflect patient location, intensity and nature of care. Costs included standard tests, consumables, staff and overheads, representing an average daily charge. This was then multiplied by length of stay to give a total ward stay cost.

Complications

The costs of extra diagnostic tests and treatment of complications were from the SMUH finance department. These were incurred over and above a standard hospital stay or episode. Drug costs were obtained from the *British National Formulary*.

Table 2 Comparison of costs of cell salvage devices

	Annual cost (£)		
	Haemonetics® Cell Saver® 5	Cobe® BRAT® 2	Fresenius CATS
Capital	2255	2416	3140
Maintenance	2377	1880	1650
Consumables	2956	4250	5150
Total	7588	8546	9940
Cost per case*	152	171	199

*Based on activity of 50 operations per annum. Annual capital costs calculated with an annuity factor of 6 per cent and a life of 8 years

Results

Clinical outcome

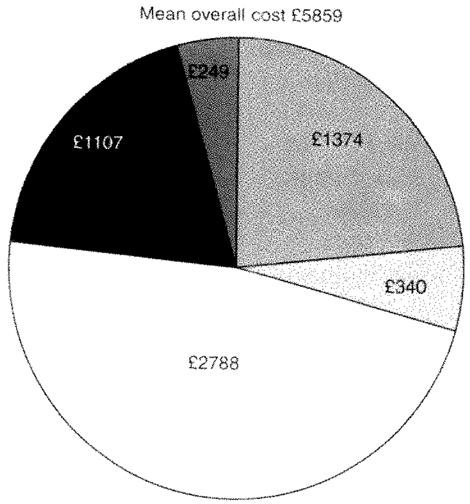
Some 197 patients were screened for the trial and 145 were included over 30 months[4]. Seventy-one patients were randomized to standard homologous transfusion and 74 to autologous transfusion. Thirty-four underwent aortobifemoral bypass and 111 had aortic aneurysm repair. The clinical results of the ATIS trial have been reported elsewhere[3,4,11]. In summary, median blood loss during operation was 1000 (i.q.r. 688–1734) ml in patients receiving homologous blood and 921 (661–1374) ml in those receiving autologous transfusion ($P = 0{\cdot}37$). The median packed red cell volume reinfused following cell salvage was 415 (i.q.r. 225–543) ml, equivalent to more than 1 unit of homologous blood as the haematocrit of reinfused red cells was approximately 65 per cent. Patients randomized to homologous transfusion received a median of 2 (i.q.r. 0–4) units of homologous blood (total 251 units), compared with 0 (0–2) units in the autologous transfusion group (total 103 units) ($P = 0{\cdot}008$). Median theatre time was 205 (i.q.r. 170–231) min for homologous and 195 (162–238) min for autologous transfusion procedures ($P = 0{\cdot}86$). There were no differences in mortality or postoperative complications. Median postoperative hospital stay was 9 (i.q.r. 7–12) days in patients having homologous transfusion and 10 (8–13) days in those receiving autologous blood ($P = 0{\cdot}17$). Median intensive care requirement was 1 (range 0–25) days in both groups ($P = 0{\cdot}89$).

Costs

Assuming an activity of 50 patients per annum and the provision of a cell salvage operator, there was no significant difference in the mean cost of treatment for homologous or autologous transfusion (£5859 *versus* £5384 respectively). The majority of the total cost was due to intensive care and ward stays: transfusion accounted for only 6 and 7 per cent of the total in homologous and autologous transfusion groups respectively (*Fig. 1*). Breakdown of the costs showed no significant differences between the transfusion strategies (*Table 3*).

Sensitivity analyses

The exclusion of a dedicated cell salvage operator had no influence on the overall results, with cost equivalence being



a Homologous transfusion policy (*n* = 71)

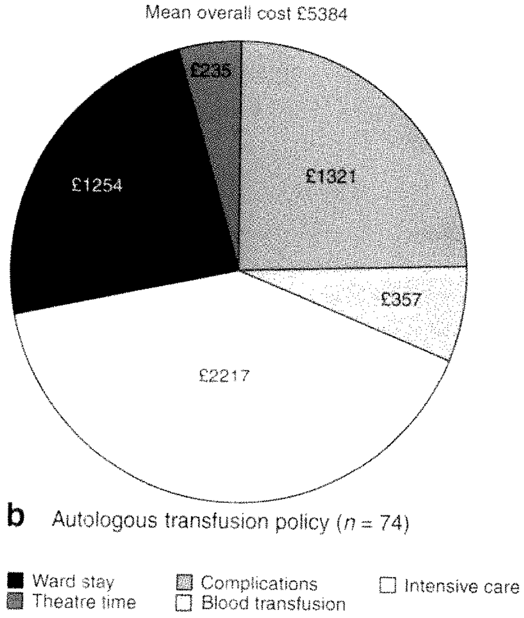


Fig. 1 Mean costs for **a** homologous and **b** autologous transfusion policies

maintained. Similarly, reducing activity to 20 operations per annum, or increasing activity to 150 operations per annum, had little impact on costs (*Table 4*). Analysis of transfusion-related costs in isolation also failed to show significant cost benefits for one particular strategy (results not shown).

Despite the differences in capital, disposable and maintenance costs for the three cell salvage devices (*Table 2*), there was no financial advantage for one system over another. Assuming all operations were performed exclusively with one system, the mean differences compared with homologous transfusion were a reduction in cost of £468 (95 per cent c.i. – £2383 to £1143) for the Haemonetics® Cell Saver® 5, £449 (– £2341 to £1325) for the Cobe® BRAT® 2, and £421 (– £2173 to £1229) for the Fresenius CATS; the latter two systems were slightly more expensive to run.

Table 3 Cost analysis: autologous *versus* homologous transfusion

	Mean difference (£)
Theatre	– 54 (– 136, 47)
Transfusion	17 (– 184, 174)
Intensive care	– 571 (– 2236, 1213)
Ward	147 (– 164, 162)
Complications	– 14 (– 86, 48)
Total	– 475 (– 2247, 1052)

Values in parentheses are 95 per cent confidence intervals. Costs based on activity of 50 operations per annum and provision of a dedicated cell salvage operator

Table 4 Two-way sensitivity analysis of activity and provision of a cell salvage operator: autologous *versus* homologous transfusion

Activity (operations per annum)	Mean difference (£)
With cell salvage operator	
20	– 321 (– 2391, 1391)
50	– 475 (– 2231, 1342)
150	– 514 (– 2315, 1147)
Without cell salvage operator	
20	– 361 (– 2254, 1274)
50	– 495 (– 2406, 1170)
150	– 555 (– 2423, 1202)

Values in parentheses are 95 per cent confidence intervals

Discussion

This study showed that the cost of aortic surgery with ANH and ICS was equivalent to that with standard homologous blood transfusion. Furthermore, there were no differences between the costs of homologous and autologous transfusion, whether or not a dedicated cell salvage operator was employed. Given the minimal cost differences between transfusion strategies demonstrated by this study, more than 1000 patients would need to be studied to demonstrate a statistically significant difference with approximately 80 per cent power (exact numbers would vary according to the level of surgical activity chosen). Although a much larger multicentre trial might ensure a fast recruitment, such numbers would represent well over a decade of activity for most hospitals individually. In this interval, changes in costs due to inflation, advances in technology and delivery of care would certainly outweigh the importance of this difference. Therefore, even assuming that a small difference in cost could be demonstrated, this would probably be irrelevant to financial planning.

Previous studies of autologous transfusion in aortic surgery generally lacked controls and assumed that the reinfusion of 1 unit of cell salvage blood equated to 1 unit of homologous blood saved[5,6,18].

The cost of aortic surgery in this study was comparable to that in previous reports from the UK[19]. The study also confirmed that the largest single expense associated with this type of operation is the time spent in intensive care[20], with transfusion costs accounting for only 6–7 per cent of the total expenditure. Not surprisingly, therefore, the use of different cell salvage devices and the inclusion of a dedicated cell salvage operator would have impacted on the overall costs only if there had been a difference in the frequency of complications or the duration of hospital stay. For the purpose of this evaluation it was necessary to make assumptions of surgical activity to attribute cell salvage costs. In reality, however, a cell salvage device would not be used exclusively for one surgical procedure. Running costs would be further diluted by cell salvage being available for ruptured aneurysms, where its value is undisputed[21].

Although some authors have reported improved outcome with autologous transfusion[22], mortality and morbidity in the present study were not influenced by transfusion technique[3,4,11].

These results are in disagreement with published theoretical modelling suggesting that cost equivalence with autologous transfusion can be achieved only by a reduction in hospital stay of up to 2 days[23]. Recent increases in the cost of homologous blood in the UK[24] have a profound influence on any such comparison.

The low recruitment rate in this trial may cast doubt on the generalizability of the results. Several issues explain this low recruitment. Not all participating centres recruited for the whole 30 months; two hospitals in particular stopped recruitment after two and five operations respectively, after the participating surgeon left in one and the provision of vascular services was halted in the other. Patients were screened for inclusion only if facilities for autologous transfusion were available on the day the operation was scheduled. Approximately three-quarters of those screened were randomized; more importantly, only ten patients (5 per cent of those screened) were rejected on the grounds of systemic co-morbidity[4].

The cost equivalence of homologous and autologous transfusion implies that the choice of transfusion strategy should be made exclusively on the basis of optimal clinical care. Concerns about availability of blood products and patient preference, however, may influence this decision.

Acknowledgements

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Vascular Surgical Society

Prospective multicentre study of the natural history of atherosclerotic renal artery stenosis in patients with peripheral vascular disease

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Background: Many patients with peripheral vascular disease have coincident renal artery stenosis. The present study characterized the natural history of the condition.

Methods: Some 98 patients (71 men) with more than 50 per cent atherosclerotic renal artery stenosis (unilateral 64, bilateral 34) were recruited prospectively. Measurements of serum creatinine, blood pressure and renal size were recorded at baseline and every 6 months, for a minimum of 2 years.

Results: Data were available for 85 patients with a minimum follow-up of 2 years. The mean age was 71 (range 51–87) years. All 52 patients with unilateral renal artery stenosis were managed conservatively (group 1); 21 of the 33 patients with bilateral disease had no intervention (group 2) and the remaining 12 had angioplasty or reconstruction (group 3). The overall mortality rate was 32 per cent at 2 years (27 patients) and this was similar in all three groups. In only three patients was death related directly to renovascular disease; coronary disease accounted for the majority of deaths. All three patients who needed dialysis died within 1 year. In survivors from groups 1 and 3 there was a significant increase in serum creatinine concentration at follow-up. Blood pressure did not increase significantly.

Conclusion: Patients with renal artery stenosis and peripheral vascular disease had a poor prognosis, but this was not directly attributable to renal failure.

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Introduction

Renal artery stenosis (RAS) is due to atherosclerosis in 90 per cent of patients[1]. It is an important cause of progressive renal failure and renovascular hypertension. Atherosclerosis is often widespread in these patients, who have concurrent cardiac, cerebrovascular and peripheral vascular disease. RAS has been observed in up to 50 per cent of patients who have angiography for peripheral vascular disease[2]. Its true prevalence is unknown, but autopsy studies show an incidence of moderate or severe RAS of 53 per cent[3].

RAS progresses if left untreated and 11 per cent of stenoses greater than 60 per cent occlude within 2 years[4]. Approximately 40 per cent of patients with critical RAS have a decrease in glomerular filtration rate of more than 25 per cent[5]. The inverse of serum creatinine has been plotted as a linear decrease over time[6,7]. Progressive deterioration in renal function appears to be common.

Recent studies have highlighted the potential role of revascularization in preventing the progression of ischaemic nephropathy and renovascular hypertension[8–10]. There is no consensus regarding selection of patients for revascularization. Few studies have demonstrated an improvement in survival compared with patients managed conservatively. The natural history of RAS remains unclear and in the clinical setting it presents a management dilemma when discovered incidentally.

The current study aimed to document the medium-term outcome in patients with RAS diagnosed during angiography for peripheral vascular disease; clinical disease progression, intervention and mortality rates were examined.

Patients and methods

This was a prospective multicentre observational study with participation from hospitals in the Joint Vascular Research

Table 1 Outcome for 85 patients with renal artery stenosis after a minimum follow-up of 2 years

	Group 1 Unilateral	Group 2 Bilateral (No intervention)	Group 3 Bilateral (Intervention)
No. of patients	52	21	12
Deaths	17 (33)	6 (29)	4 (33)
Dialysis	2	0	1
Mean no. of antihypertensive drugs			
Baseline (All)	1·08	1·00	1·64
Baseline (Survivors)	0·89	1·00	1·71
Follow-up (Survivors)	0·97	1·13	1·67

Values in parentheses are percentages. All patients who had dialysis died

Group. Between 1994 and 1998, 98 patients were enrolled for longitudinal follow-up.

All patients had undergone angiography for peripheral vascular disease and were found incidentally to have significant RAS (greater than 50 per cent). Bilateral RAS was defined as either significant disease in both renal arteries, significant stenosis and contralateral occlusion or significant RAS in the presence of a solitary kidney. Measurements of renal size, serum creatinine and blood pressure, and use of antihypertensive drugs were recorded at baseline and at 6-month intervals. Renal artery intervention was undertaken at the discretion of the managing clinician.

Patients were classified into three groups for analysis: those with unilateral renal artery disease managed without intervention (group 1), patients with bilateral disease managed without intervention (group 2) and patients with bilateral disease who had intervention (group 3).

Endpoints were death or status at termination of the study. Statistical analysis was undertaken using SPSS 9.0 for Windows (SPSS, Chigaco, Illinois, USA).

Results

Ninety-eight patients (71 men) were recruited; 34 had bilateral RAS and the 64 unilateral stenoses were equally distributed between right and left sides. Complete data were available for 85 patients with a minimum follow-up of 2 years (mean 2·5 years). The mean age of the patients was 71 (range 51–87) years. All 52 patients with unilateral disease (group 1) were managed conservatively. Of the 33 patients with bilateral disease, 21 were managed without intervention (group 2) but the remaining 12 had intervention with angioplasty or surgery (group 3).

All 12 patients with bilateral disease who had intervention were men, with a mean age of 72 years. Most patients had intervention for salvage of renal function but one angioplasty was done for successful control of renovascular hypertension and one for flash pulmonary oedema. Eleven endovascular procedures were performed comprising nine angioplasties (one failure) and one bilateral renal artery stenting. Four kidneys had open revascularization by surgery (one bilateral aortorenal bypass during elective aortic aneurysm repair, one left aortorenal bypass and one left ileorenal bypass).

After 2 years' follow-up, the overall mortality rate was 32 per cent; this was similar in the three groups (*Table 1*). In only three patients was death related directly to renovascular disease; coronary disease accounted for the majority of other deaths. All three patients who required dialysis died within 1 year. The mean number of antihypertensive drugs did not change significantly with follow-up. Renal size measured by transabdominal ultrasonography was found to be inconsistent owing to interobserver variability. Median blood pressure in survivors did not change significantly with follow-up (*Fig. 1*). In survivors from groups 1 and 3 there was a significant increase in serum creatinine at follow-up ($P = 0.002$ and $P = 0.012$ respectively, Wilcoxon signed rank test for paired observations) (*Fig. 2*).

Nine of the 33 patients with bilateral disease had contralateral renal artery occlusion and two had a previous nephrectomy (one for renal cell carcinoma and one because of renal sepsis). Of the nine with contralateral occlusion, three patients underwent intervention (two angioplasties and one surgical bypass after failed angioplasty) for salvage of renal function and one underwent angioplasty for flash pulmonary oedema. The mean serum creatinine concentration at the time of intervention was 350 μmol/l. Overall five of these nine patients died, three despite intervention. Only one death was related to end-stage renal failure. The two patients with a previous nephrectomy were alive at 2·5 and 2 years without any intervention.

Kaplan–Meier survival analysis was performed. There was no significant difference in overall survival when patients with unilateral RAS were compared with all patients with bilateral RAS (log rank test statistic 0·01, $P = 0.91$, 1 d.f.) (*Fig. 3*). Survival analysis for patients

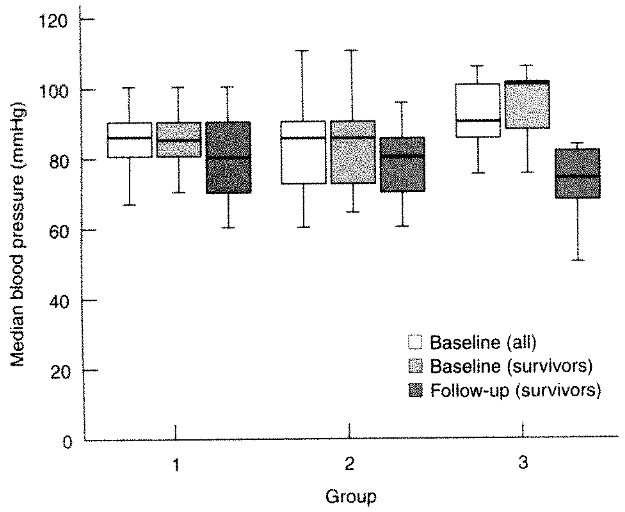

Fig. 1 Median diastolic blood pressure during follow-up in 85 patients with renal artery stenosis (RAS). Group 1, unilateral RAS; group 2, bilateral RAS without intervention; group 3, bilateral RAS with intervention. Horizontal bars, boxes and error bars represent median, quartiles and extreme values respectively

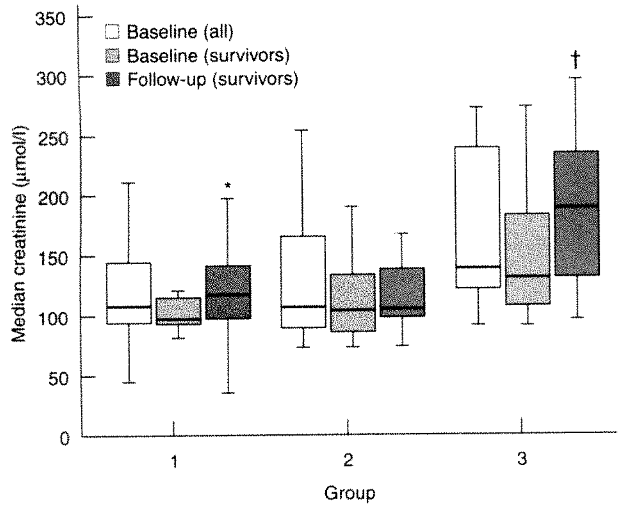

Fig. 2 Median serum creatinine during follow-up in 85 patients with renal artery stenosis (RAS). Group 1, unilateral RAS; group 2, bilateral RAS without intervention; group 3, bilateral RAS with intervention. Horizontal bars, boxes and error bars represent median, quartiles and extreme values respectively. $^{*}P = 0{\cdot}002$, $^{\dagger}P = 0{\cdot}012$ *versus* baseline (survivors) (Wilcoxon signed rank test for paired observations)

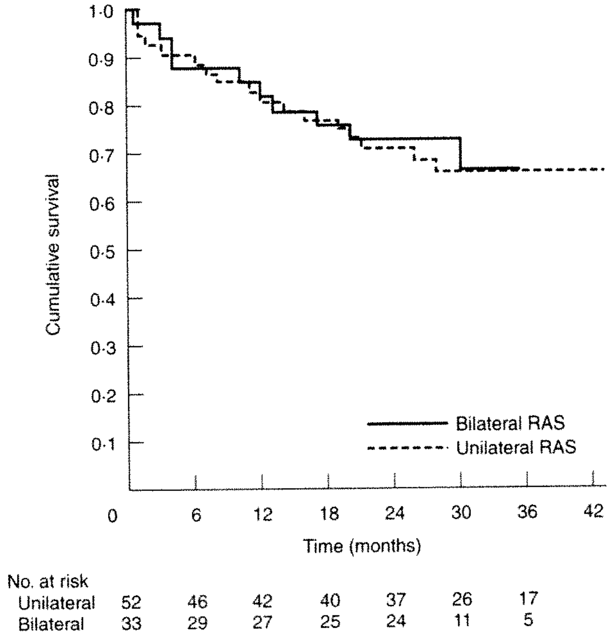

Fig. 3 Kaplan–Meier survival plots for 85 patients with renal artery stenosis, comparing outcome for those with unilateral and bilateral disease

managed medically revealed that there was no significant difference in survival between 52 patients with unilateral and 21 patients with bilateral RAS (log rank test statistic 0·05, $P = 0{\cdot}82$, 1 d.f.). Analysis of patients with bilateral RAS demonstrated no significant difference in survival between 12 patients who had intervention and 21 patients who were managed medically (log rank test statistic 0·07, $P = 0{\cdot}75$, 1 d.f.).

Discussion

The association between peripheral vascular disease and RAS has been described before[8,11]. The male preponderance (72 per cent) in the present study reflects the higher percentage of men undergoing angiography for peripheral vascular disease. Other studies of angioplasty reveal sex ratios of 51–59 per cent[12,13].

No patient with a unilateral stenosis underwent intervention. The patients under study here were primarily under the care of a vascular surgeon and RAS was an incidental finding. Scoble *et al.*[11] reported an intervention rate of 30 per cent for unilateral RAS; their intervention rate for bilateral disease was twice that in the present study. These patients were under the care of a renal physician and presumably presented with renal impairment; they had a higher baseline mean creatinine than patients in the present study.

Patients with bilateral stenosis who underwent intervention had a higher median serum creatinine level at baseline than those treated medically. The intervention group probably had a negative selection bias, whereby patients with more severe demonstrable renal disease were treated. This may explain the similar mortality rate in the two groups, despite the potential advantage of intervention.

Patients with contralateral renal artery occlusion had a slightly higher mortality rate (five of nine), unaffected by intervention, as has been described previously[8]. The higher mortality rate may reflect an advanced state of systemic vascular disease or poor renal reserve in the ipsilateral kidney.

The poor overall survival demonstrated in this study is echoed by a recent retrospective study of 68 similar patients after a mean follow-up of 39 months[14]. The majority of deaths were unrelated to renal causes. Vascular co-morbidity seemed to be the major determinant of long-term outcome. The burden on renal replacement services from the present cohort was not significant; the survivors appeared to have stable blood pressure and only moderate deterioration in renal function.

Approximately one-third of patients with peripheral arterial disease and coexisting renal arterial disease in this study were dead within 2 years. Intervention to improve renal artery blood flow did not appear to have any impact on this outcome. Furthermore, although patient numbers were small, intervention did not appear to improve overall renal function, as assessed by serum creatinine measurement. Only patients with bilateral renal arterial disease in whom intervention was performed showed clinically significant deterioration in renal function after follow-up of 2 years. However, many of these patients initially had the worst renal function among all patients in the present study.

A randomized controlled trial is still needed to determine whether renal revascularization confers any long-term benefit over medical management. There are a large number of confounding variables, and it is possible that intervention will only benefit a subgroup of patients.

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Potential role of endothelin 1 in ischaemia-induced angiogenesis in critical leg ischaemia

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Background: Ischaemia-induced angiogenesis occurs in critical leg ischaemia (CLI) and endothelin (ET) 1 may be involved in this process. The aim of this study was to quantify microvessels and study ET receptor expression and distribution in critically ischaemic leg muscle.
Methods: Leg muscle biopsies were taken from 12 patients with CLI and 12 patients with no leg ischaemia. Microvessels were identified immunohistochemically on muscle sections, and the number of immunopositive cells was quantified. ET_A and ET_B receptor messenger RNA (mRNA) expression was studied using real-time quantitative reverse transcriptase–polymerase chain reaction, and receptor binding was localized and assessed by *in vitro* autoradiography.
Results: The number of microvessels in CLI muscle biopsies was 2·6 times higher than that in controls ($P < 0{\cdot}01$). ET_B receptor mRNA expression and binding were significantly increased in CLI tissue ($P < 0{\cdot}05$), while ET_A receptor levels were not significantly raised. High-resolution autoradiography showed that ET receptor binding was associated with microvessels.
Conclusion: Angiogenesis occurs in CLI and raised ET_B receptors within the muscle were associated with microvessels, suggesting that ET-1 may mediate angiogenesis via these receptors in critically ischaemic muscle.

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Introduction

Tissue hypoxia is a strong physiological stimulus for angiogenesis[1] and ischaemia-induced angiogenesis has been shown to occur in animal models of skeletal muscle ischaemia[2]. Autocrine and paracrine substances play an important role in the process of angiogenesis, and many endogenous proangiogenic and antiangiogenic factors have been identified[1].

Endothelin (ET) 1 is a 21-amino acid peptide released by a variety of cells, including endothelial cells, with potent vasoconstrictor activity[3]. It also has mitogenic and proliferative properties on endothelial and vascular smooth muscle cells[4,5], and may therefore be a potential angiogenic factor. In addition, ET-1 production is stimulated by hypoxia[6,7] suggesting that it may promote angiogenesis under such conditions. Two main ET-1 receptor subtypes have been identified, ET_A and ET_B receptors[8], which mediate the various effects of ET-1[6].

In patients with critical leg ischaemia (CLI), arterial insufficiency results in chronic muscle ischaemia and hypoxia. Increased ET-1 immunoreactivity has been demonstrated in muscle biopsies from these patients, suggesting that ET-1 may play a role in the pathophysiology of CLI[9].

The aim of this study was to quantify microvessels, and to study the expression and distribution of ET receptors in critically ischaemic and non-ischaemic leg muscle.

Patients and methods

Twelve patients undergoing major lower-limb amputation for CLI and 12 surgical patients with no significant peripheral vascular disease (controls) were studied. In the control group, six patients had total knee replacement for osteoarthritis and six had coronary artery bypass graft surgery, enabling access to the leg muscles during surgery. Significant peripheral vascular disease in the control group was excluded by clinical history, examination and ankle : brachial pressure index (ABPI) measurement (patients with an ABPI of less than 0·9 were excluded). Local ethics committee approval and informed consent from each patient was obtained.

The mean age of the patients with CLI was 76 (range 59–86) years. There were eight men and four women, and two were current smokers. In the control group, the mean age was 73 (range 59–85) years; there were four women and one smoker.

Muscle biopsies

Open muscle biopsies were taken intraoperatively from both groups of patients on exposure of the leg muscles. Tissue was frozen immediately on dry carbon dioxide for autoradiography, and in liquid nitrogen for reverse transcriptase–polymerase chain reaction (RT–PCR), and stored at – 70°C until use. In addition, tissue was also fixed by immersion in 10 per cent neutral-buffered formalin for immunohistochemistry.

For autoradiography, 10-μm serial cryostat sections were cut at –25°C and three sections from each muscle biopsy were sequentially thaw-mounted on to polylysine-coated microscope slides. Slides were coded for blinded analysis and stored at – 70°C until used. Similarly, formalin-fixed tissue was embedded in paraffin blocks; 6-μm sections were cut on a microtome, mounted on to polylysine-coated slides and coded.

Immunohistochemistry

Immunohistochemistry was performed using the avidin–biotin complex (ABC) peroxidase method. Microvessels were identified on muscle sections using monoclonal antibodies against platelet–endothelial cell adhesion molecule 1 (CD31; Dako, Glostrup, Denmark) to identify endothelial cells and smooth muscle α-actin (α-SMA; Dako) to identify vascular smooth muscle. Briefly, sections were deparaffinized and rehydrated. For CD31, antigen retrieval was carried out by placing the sections in citrate buffer 0·01 mmol/l and microwaving for 5 min[10]. Endogenous peroxidase activity was inhibited by immersion in 0·5 per cent hydrogen peroxide in methanol for 10 min. Sections were then incubated in 1 per cent normal horse serum for 20 min to block non-specific binding, followed by primary antibody (anti-CD31 or anti-α-SMA diluted 1 : 100) in phosphate-buffered saline (PBS) for 1 h at room temperature. Sections were rinsed in PBS and incubated with biotinylated secondary antibody for 30 min, followed by incubation in avidin–biotinylated horseradish peroxidase complex (Vectastain Elite® ABC kit; Vector Laboratories, Burlingame, California, USA) for 30 min. Peroxidase activity was revealed by applying diaminobenzidine for 10 min. Sections were counterstained with haematoxylin, prepared for microscopic examination, and examined and photographed using an Olympus BX 50® (Olympus Optical, London, UK) microscope.

Quantification of microvessels

Microvessels were quantified in a blinded fashion by counting the number of CD31-positive cells within a 100 × 150 μm grid at × 200 magnification. Five random areas per section and three sections per biopsy were analysed.

Endothelin receptor subtype messenger RNA expression

Gene expression was studied using quantitative RT–PCR with real-time detection using Taqman® probes (Applera Europe B.V., Rotkreuz, Switzerland). Total RNA was isolated from homogenized muscle biopsies using TRIzol® reagent (Invitrogen Life Technologies, Paisley, UK)[11,12]. Equal amounts of total RNA from each sample were treated with RQ1 RNase-free DNase (Promega, Madison, Wisconsin, USA) and then reversed transcribed into complementary DNA (cDNA) using random hexamer primers (Roche, Mannheim, Germany), Moloney murine leukaemia virus reverse transcriptase (Invitrogen Life Technologies) and deoxyribonucleoside 5′-triphosphates (dNTP mix; Promega) according to the manufacturers' instructions.

The cDNA sample was then used for real-time quantitative PCR, performed with the ABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, California, USA) as previously described[13,14]. Glyceralde-hyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous control. The primer and probe sequences used are shown in *Table 1*. The thermal cycling conditions were as follows: 50°C for 2 min twice and 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 65°C for 1 min. Samples were analysed in triplicate.

Quantitative values were obtained from the threshold cycle number at which the increase in signal associated with an exponential growth of PCR products was detected (C_T). Each sample was normalized to its content of GAPDH (ΔC_T). ΔC_T values were used for statistical analysis. In

Table 1 Primer and probe sequences used for quantitative reverse transcriptase–polymerase chain reaction

Gene		Sequence
GAPDH	Forward primer	CCAGGAAATGAGCTTGACAAAGTT
	Reverse primer	CTCCTCCACCTTTGACGCTG
	Probe	TCGTTGAGGGCAATGCCAGCC
ET_A	Forward primer	ACCACAACACAGACCGGAGC
	Reverse primer	GGGAGTACCGAGGAGTGCTTC
	Probe	CCATAAGGACAGCATGAACTGACCACCC
ET_B	Forward primer	GTGCCAAGGACCCATCGA
	Reverse primer	GCACGAACACAAGGCAGGA
	Probe	TCAAGGAGACTTTCAAATACATCAACACGGTTGT

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ET, endothelin

addition, results were expressed as *N*-fold differences in target gene expression between CLI muscle samples and control biopsies, determined as follows:

$$N\,\text{target} = 2^{-(\Delta C_T\ \text{sample}\ -\ \Delta C_T\ \text{control})}.$$

Autoradiography

ET-1 receptors were identified using ^{125}I-radiolabelled ET-1[15]. ET_A and ET_B binding sites were assessed using the subtype-selective radioligands ^{125}I-labelled PD151242[16] and ^{125}I-labelled BQ3020[17] respectively, as described previously[18].

Autoradiographs were generated by exposing incubated tissue to Hyperfilm ^{3}H (Amersham Pharmacia Biotech, Amersham, UK) and used for densitometric analysis of receptor binding using a Bio-Rad GS-700 imaging densitometer with Molecular Analyst Software (Bio-Rad Laboratories, Hercules, California, USA). Binding was expressed in terms of radioligand bound per unit area (disintegrations per minute (d.p.m.) per square millimetre), calculated from standard curves generated by ^{125}I-microscales (Amersham Pharmacia Biotech) that were co-exposed with tissue sections. Specific binding was calculated by subtracting non-specific from total binding.

High-resolution autoradiography was performed using K2 nuclear emulsion (Ilford, Mobberley, UK) under darkroom conditions[19]. Slides were stored in lightproof boxes for up to 8 days at 4°C. Emulsion was then processed following the manufacturer's instructions. Underlying tissue was stained with haematoxylin and eosin for histological examination. Sections were viewed on an Olympus BX 50® microscope under bright and dark field illumination, and photographed where appropriate.

Statistical analysis

Results are presented as median values with 95 per cent confidence intervals. The two-tailed, non-parametric Mann–Whitney *U* test was performed using Prism 3.0 (GraphPad Prism 3·0®, San Diego, California, USA) with statistical significance inferred at $P < 0{\cdot}05$.

Results

Immunohistochemical identification of microvessels

CD31 and anti-αSMA immunostaining co-localized, confirming the identity of microvessels. Microvessels appeared more abundant in ischaemic muscle biopsies than control muscle (*Fig. 1*). In addition, prominent clusters of microvessels were observed in ischaemic muscle but not in control tissue (*Fig. 2*).

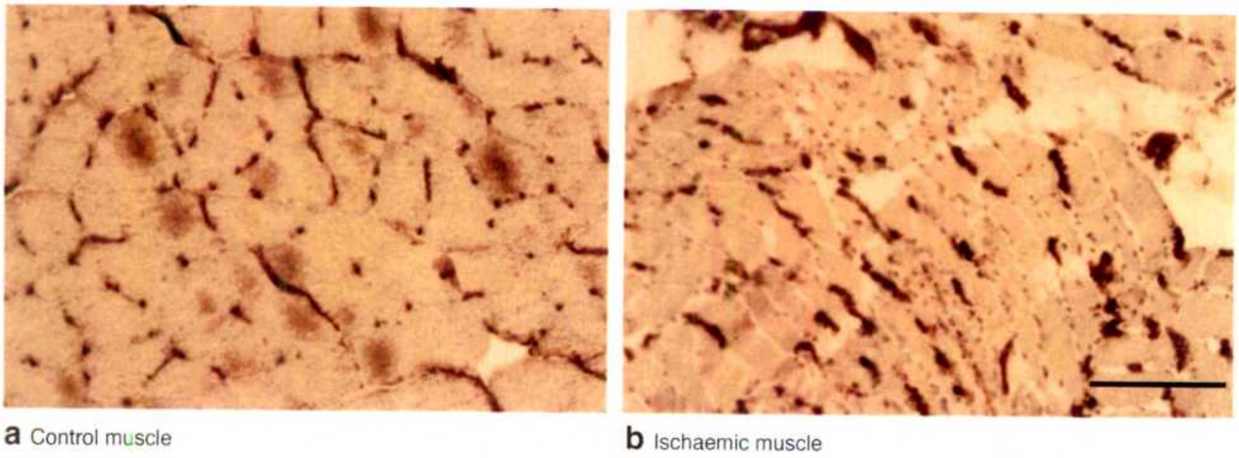
Quantification of microvessels

The median number of CD31-immunopositive cells per grid area was higher in ischaemic muscle biopsies (9·8 (8·4 to 12·4) microvessels per grid) than in controls (3·8 (3·6 to 4·4) microvessels per grid) ($P < 0{\cdot}01$, $n = 12$) (*Fig. 3*). The intersubject variability was small, with no significant difference in microvessel numbers between sections from the same patient ($P > 0{\cdot}05$, Friedman test).

Endothelin receptor subtype messenger RNA expression

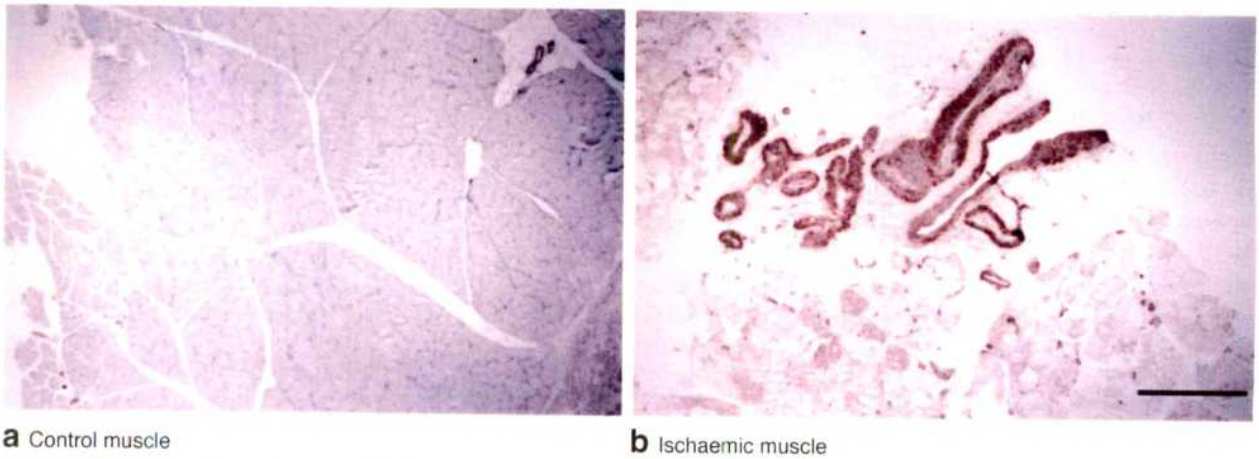
ΔC_T for ET_A receptors was 3·7 (2·4 to 4·5) in CLI muscle biopsies and 5·7 (3·7 to 7·4) in control biopsies, $N = 3{\cdot}86$, which represented a non-significant increase in ET_A receptor mRNA ($P = 0{\cdot}10$, $n = 7$).

ΔC_T for ET_B receptors was 11·1 (9·7 to 12·0) in CLI muscle biopsies and 12·7 (12·0 to 13·2) in control biopsies, which represented a 3·03-fold increase in ET_B receptor mRNA level in ischaemic muscle ($P < 0{\cdot}01$, $n = 7$) (*Fig. 4*).


a Control muscle

b Ischaemic muscle

Fig. 1 CD31 immunoreactivity in **a** control and **b** ischaemic muscle. Increased CD31 immunoreactivity (brown staining) was seen in ischaemic muscle compared with control muscle. Such slides were used for the quantitative assessment of microvessels. Scale bar = 100 µm


a Control muscle

b Ischaemic muscle

Fig. 2 Antismooth muscle α-actin (α-SMA) immunoreactivity in **a** control and **b** ischaemic muscle. Clusters of microvessels were identified by anti-α-SMA immunostaining in ischaemic muscle but not in controls. These may represent areas of neovascularization. Scale bar = 250 µm

Autoradiographic localization of endothelin 1 binding

On examination of autoradiographs, binding of ^{125}I-radiolabelled ET-1 to both CLI and control muscle biopsies was observed (not shown). Using subtype-selective radioligands, both ET_A and ET_B receptors were found to be involved, and clusters of ET_A and ET_B receptor binding were observed within the muscle sections. On densitometric analysis, non-specific binding accounted for less than 10 per cent of total binding. ^{125}I-labelled BQ3020 binding to ET_B receptors was significantly increased in CLI compared with control muscle (4·93 (3·32 to 6·28) *versus* 1·67 (1·33 to 3·34) $\times 10^3$ d.p.m. per mm^2; $P < 0·05$) whereas ^{125}I-labelled PD151242 binding to ET_A receptors was not significantly altered (3·75 (3·08 to 5·06) *versus* 2·45 (1·82 to 3·57) $\times 10^3$ d.p.m. per mm^2; $P = 0·06$, $n = 12$ *versus* $n = 10$) (*Fig. 5*).

Using high-resolution autoradiography, a large proportion of the discrete clusters of ET_A and ET_B receptor binding was found to be associated with microvessels within the muscle section (*Fig. 6*).

Discussion

Angiogenesis occurs in hypoxic conditions such as in tumours, regions of wound healing and atherosclerotic

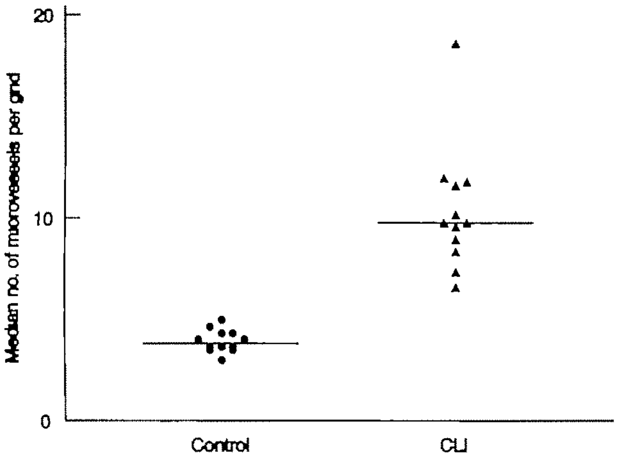


Fig. 3 Microvessel density, quantified by counting the number of CD31-positive cells within a 0·015-mm^2 grid. Values for individual patients are shown and horizontal bars represent group median values. Microvessel density was higher in chronic leg ischaemia (CLI) muscle biopsies than in controls ($P < 0·01$, Mann–Whitney *U* test)

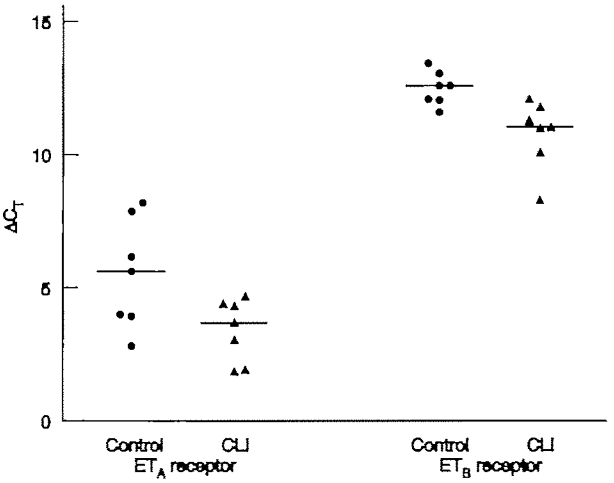


Fig. 4 Endothelin (ET) receptor messenger RNA (mRNA) expression. ΔC_T values obtained from real-time polymerase chain reaction (PCR) are the threshold cycle numbers at which an exponential increase in PCR products was detected; a lower ΔC_T value represents a higher mRNA level in the sample. Values for individual patients are shown and horizontal bars represent group median values. ΔC_T for ET$_A$ receptors was not significantly different between chronic leg ischaemia (CLI) and control muscle biopsies ($P = 0·10$), while that for ET$_B$ receptors was significantly less in ischaemic muscle biopsies ($P < 0·05$) (Mann–Whitney *U* test)

plaques as a compensatory mechanism to improve tissue perfusion[1]. In skeletal muscle ischaemia, increased microvessel density has been shown in animal models[2]. However, in these models the onset of ischaemia is usually subacute, induced by a focal lesion. In humans, peripheral vascular disease is the commonest cause of leg ischaemia and has a chronic course.

To investigate microvessel density and the ET-1 pathway in chronically ischaemic muscle, muscle biopsies taken from patients with CLI were studied. Raised ET-1 immunoreactivity has previously been found in muscle biopsies from these patients, with ET-1 immunostaining associated with microvessels within the muscle[9]. ET-1 via its potent vasoconstrictor activity may increase microvessel tone and exacerbate ischaemic damage. However, other ET-1-mediated effects are also likely to play a role in CLI.

The ability of ET-1 to stimulate endothelial cell proliferation and migration and vascular smooth muscle cell proliferation suggests that this peptide may be able to stimulate angiogenesis[20,21]. In addition, ET-1 protects endothelial cells against apoptosis[22] and may play a further role in the maintenance of angiogenesis. Evidence for a role of ET-1 in angiogenesis includes its presence in well vascularized tumours such as gliomas, where ET-1 levels correlate with tumour vascularity and malignancy[23]. This is supported by the finding of ET-1 binding sites on vessels of pulmonary tumours[24], areas of neovascularization in atherosclerotic coronary arteries[25] and in failed saphenous

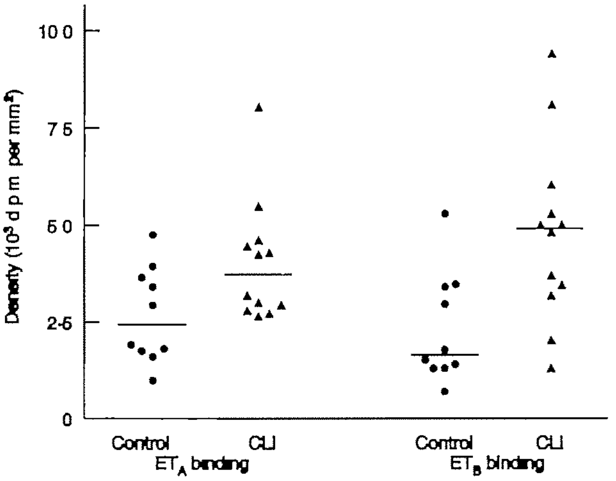


Fig. 5 Densitometric analysis of endothelin (ET) receptor subtype binding. Values for individual patients are shown and horizontal bars represent group median values. ET$_A$ receptor binding was not significantly increased ($P = 0·06$), whereas ET$_B$ receptor binding was significantly greater in chronic leg ischaemia (CLI) muscle biopsies compared with controls ($P < 0·05$) (Mann–Whitney *U* test)

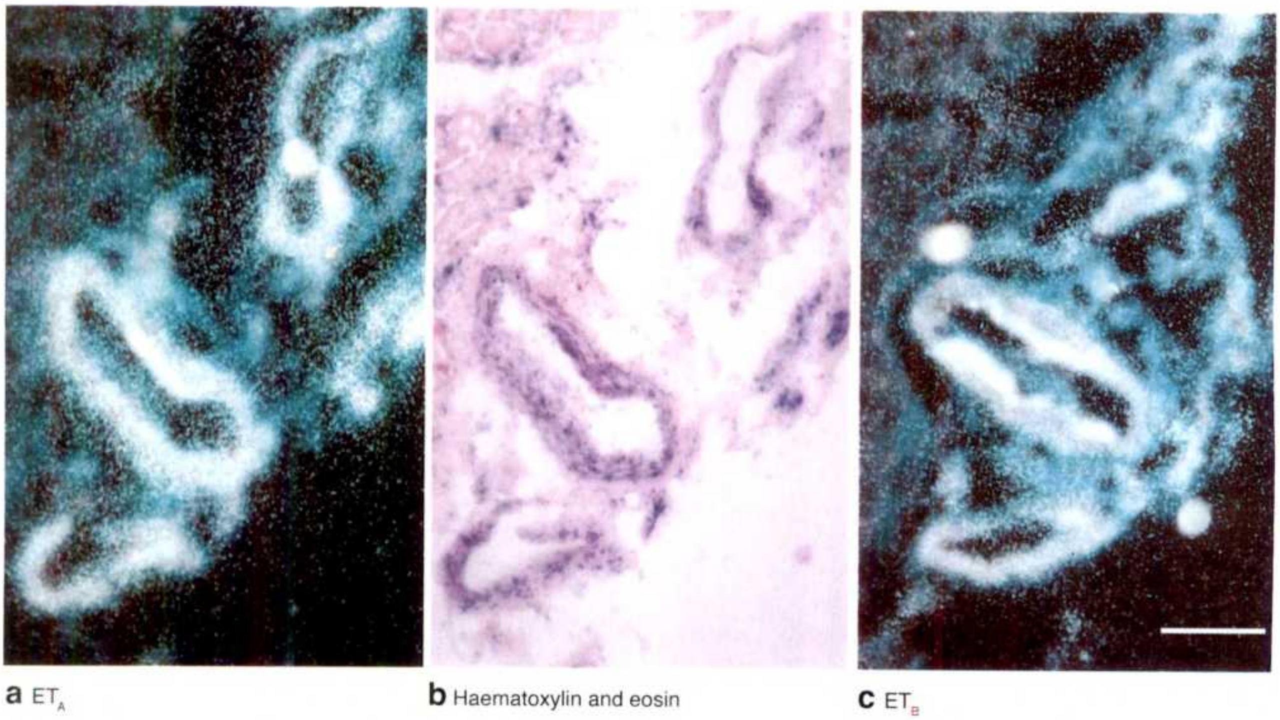


Fig. 6 High-resolution autoradiograph of endothelin (ET) receptor subtype binding to microvessels in ischaemic muscle biopsy. ET_A (**a**) and ET_B (**c**) receptor binding (seen as white grains) was localized to microvessels within muscle biopsies. **b** Underlying tissue stained with haematoxylin and eosin. Scale bar = 100 μm

vein grafts[26]. ET-1 has been shown to promote human umbilical vein endothelial cell proliferation, migration and invasion *in vitro*[27], and to induce new vessel formation in *in vivo* models of angiogenesis[27,28].

ET-1 acts via two receptor subtypes. The ET_A receptor plays a predominant role in its vasoconstrictor activity, while endothelial cell migration and proliferation have been shown to be mediated by the ET_B receptor[29]. The selective ET_B receptor antagonist BQ788 inhibits ET-1-induced endothelial cell migration *in vitro*[20,27], supporting the suggestion that ET-1-induced angiogenesis is predominantly mediated via ET_B receptors.

In this study, increased microvessel density was found in muscle biopsies from patients with CLI, and ET_A and ET_B receptor binding was demonstrated on these microvessels. Receptor mRNA levels and binding were both increased in CLI muscle biopsies; the ET_B receptor, in particular, was significantly upregulated. While these findings suggest that angiogenesis occurs in CLI and that ET-1 may play a role in this process via the ET_B receptor, the mechanisms involved are likely to be complex, involving other factors that may act synergistically with, or be regulated by, ET receptor-mediated mechanisms. Further studies are required to clarify the role of ET-1 and its interaction with other factors in this process.

Control biopsies for this study were taken from surgical patients in whom intraoperative access to the leg muscles was possible. However, while peripheral vascular disease was excluded in these patients, they represented a heterogeneous group. Although no significant difference in skeletal muscle microvascularization and the ET-1 pathway was observed within the group in this study, subgroup analysis of larger numbers of controls may reveal such differences.

Manipulation of the ET-1 pathway may have potential therapeutic benefits in patients with peripheral vascular disease. Identification of the ET receptor subtype that is important clinically would allow specific ET-1-induced effects to be targeted pharmacologically. For example, receptor subtype-specific antagonists targeting potentially vasoconstrictor ET_A receptors may be used to improve vessel tone in these patients while preserving potentially beneficial ET_B-related processes such as angiogenesis.

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Vascular Surgical Society

Persistent popliteal fossa reflux following saphenopopliteal disconnection

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Background: This was a retrospective assessment of the technical adequacy of saphenopopliteal disconnection (SPD).

Methods: Patients scheduled for SPD underwent preoperative colour-coded duplex imaging to localize the saphenopopliteal junction (SPJ). Operations were conducted with the patients under general anaesthesia in the prone position with planned full popliteal fossa exposure. Patients were reimaged 6 weeks after operation.

Results: Some 69 patients had surgery over 4 years; complete data were available for 59 (27 men and 32 women, median age 55 (range 27–78) years). There were eight staged bilateral procedures. Postoperative duplex scans identified 23 (39 per cent) with ideal results (incompetent SPJ successfully disconnected), 12 (20 per cent) with satisfactory results (incompetent SPJ disconnected successfully but persisting venous reflux in superficial veins), eight duplex failures (14 per cent) (previously identified incompetent SPJ persisted but was competent after operation) and 13 surgical failures (22 per cent) (incompetent SPJ completely missed during surgery). There were three major postoperative complications (5 per cent) (two deep vein thromboses and one popliteal vein injury) and one patient suffered a sural nerve palsy.

Conclusion: Despite preoperative duplex localization of the SPJ, SPD proved an unreliable technique in this series.

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Introduction

Saphenopopliteal disconnection (SPD) as treatment for varicose veins is a technically demanding operation with a higher recurrence rate than that after saphenofemoral disconnection[1–3]. Flush ligation of the saphenopopliteal junction (SPJ) is necessary for a durable procedure[4]. Failure of identification of the SPJ is a common cause of recurrent short saphenous varices, which occur after 30 per cent of operations[2,3].

The aim of this retrospective study was to review the technical success of SPD in patients with short saphenous vein (SSV) varices as judged by preoperative and postoperative colour-coded duplex imaging.

Patients and methods

This study was a review of a series of patients who underwent SPD by one consultant vascular surgeon. Data were collected from computer-based clinic letters, operative notes and duplex scan results.

Between January 1998 and August 2001, 110 patients (118 legs) were found to have reflux at the SPJ in the one-stop vascular clinic. After counselling, 69 patients with SPJ incompetence underwent SPD. Complete data were available for analysis in 59 patients. There were 27 men and 32 women, median age 55 (range 27–78) years. In eight patients, staged bilateral procedures were undertaken. Surgical indications are shown in *Table 1*.

All patients had preoperative colour-coded venous duplex imaging (Sonos 2000; Hewlett Packard, Bracknell, UK). Examinations were conducted with the patient standing and reflux was defined as reversed flow for more than 2 s following manual calf squeeze. The SPJ was marked before operation in relation to the popliteal skin crease and the midline of the popliteal fossa.

All patients received a single preoperative dose of a low-molecular-weight heparin. Surgery was performed under

Table 1 Surgical indications for saphenopopliteal disconnection

	n
Venous ulcer	10
LDS or preulcer skin changes	9
Phlebitis	3
Eczema	3
Bleeding	1
Recurrent SSV varices	3
Uncomplicated (cosmetic)	31

One patient had more than one indication. LDS, lipodermatosclerosis; SSV, short saphenous vein

Table 2 Preoperative duplex findings

	n
Single incompetent SPJ	46
Double SPJ (one incompetent)	4
Triple SPJ (one incompetent)	1
Incompetent PTP	4
Incompetent PTP + incompetent SPJ	1
Persistent SPJ (recurrent cases)	3

SPJ, saphenopopliteal junction; PTP, posterior thigh perforator

Table 3 Duplex findings in failed operations 6 weeks after surgery

	n
Missed single incompetent SPJ	9
Failed redo surgery	2
Missed incompetent junction in a multiple junction system	1
Missed posterior thigh perforator	1

SPJ, saphenopopliteal junction

general anaesthesia with the patient prone. The popliteal fossa was explored formally through an incision centred over the duplex-predicted site of the SPJ. The SPJ, popliteal artery and major nerves were identified, and the junction was doubly flush ligated. The SSV and its varicose tributaries were avulsed through multiple stab incisions. Stripping of the SSV was not performed routinely. Patients who also had long saphenous varicose veins had standard saphenofemoral disconnection, stripping of the long saphenous vein to knee level and multiple avulsions.

Postoperative duplex imaging was conducted 6 weeks after surgery. The same consultant performed all duplex scans and operations. Preoperative and postoperative duplex results were compared. Clinical recurrence and patient satisfaction were not examined in this study.

Results

Preoperative venous duplex scan results are shown in *Table 2*. An unexpected anatomical variant was encountered on five occasions (9 per cent), with or without concomitant SPJ incompetence. In these legs, an anatomically normal SPJ was identified in the vicinity of the knee crease, with an additional incompetent posterior thigh perforating vein connected to the popliteal vein at least 2 cm above the SPJ. These junctions were frequently located 5–10 cm above the knee crease and usually lateral to the midline of the popliteal fossa.

Based on the postoperative venous duplex scan, patients were divided into four groups. Twenty-three patients (39 per cent) had an ideal result: the incompetent SPJ was successfully disconnected with no duplex evidence of residual superficial vein reflux. Twelve (20 per cent) had a satisfactory result: the incompetent SPJ was successfully disconnected, but duplex imaging showed residual superficial incompetence without deep to superficial reflux. Eight (14 per cent) had duplex failure: a previously identified, apparently incompetent junction persisted, but was found to be competent at follow-up scan. Thirteen (22 per cent) had an incompetent SPJ missed completely during surgery. Three patients had complications and were not included in one of the four groups. The overall success in achieving SPD was 59 per cent (23 'ideal' and 12 'satisfactory' results), but failure was unambiguous in 22 per cent (*Table 3*).

There was no obvious learning curve. Considering only whether the targeted SPJ had been ligated (duplex failure + unambiguous failure), in the first year of the study there were four failures in seven operations (57 per cent). In the following 3 years failure rates were six in 16 (38 per cent), six in 19 (32 per cent) and six in 17 (35 per cent). While the first year was the worst, there has been no consistent improvement subsequently.

Operations were more likely to be successful in patients with uncomplicated varicose veins (27 of 31) than in those with venous ulceration or preulcerative changes (12 of 19) ($P < 0{\cdot}01$, χ^2 test).

Three patients developed a major postoperative complication (5 per cent). Two had a deep vein thrombosis within 10 days of surgery. This was confirmed by ascending contrast venography and treated medically. Both recovered fully with no evidence of persisting deep vein thrombosis or incompetence. In both the incompetent SPJ has been eliminated. A third patient developed uncontrollable bleeding from the popliteal fossa during surgery, which ultimately required ligation of a short segment of the popliteal vein. Immediate postoperative ascending venography confirmed the injury with evidence of a tributary circulation bypassing the injured segment. This patient was anticoagulated for 3 months and has been advised to wear compression stockings, but remains symptom free 6 months after surgery. Follow-up duplex imaging revealed a patent, competent deep system and no evidence of deep to superficial reflux. One patient developed delayed-onset sural nerve palsy.

Discussion

There is ample literature concerning the outcome of surgery for long saphenous varicose veins. The outcome following surgery for varicose veins that originate from the short saphenous system is under-represented. A literature search failed to identify any previous investigations that systematically examined the technical adequacy of SPD. Nevertheless, the failure of localization and flush ligation of the SPJ is held to be a chief cause of recurrence of varicose veins. Tong and Royle[5] studied 70 patients with recurrent varicose veins following SSV surgery. Recurrence was attributed to an intact incompetent SPJ in 28 per cent of procedures.

The single most important finding in this study was the high rate of technical failure. These findings are at variance with a small study in which the outcome was satisfactory with popliteal fossa stab incision alone[6]. Despite preoperative duplex imaging and the attention of a specialist surgeon, between a quarter and a fifth of attempted SPDs failed to reverse deep to superficial reflux in the popliteal fossa.

The dominant cause of failure in this study was a missed single incompetent SPJ (nine of 13 technical failures), which occurred despite preoperative localization with duplex imaging and a convincing SPJ disconnection during surgery. This might be explained by the variability of the SSV termination[7], but such a hypothesis presupposes that preoperative imaging underestimated the complexity of the SPJ in a large number of patients. There was an ambiguous group that comprised patients labelled 'duplex failures' representing either misdiagnosis of reflux in a competent SPJ (i.e. incorrect preoperative scan leading to failed but unnecessary SPD) or failure to identify a double SPJ with one incompetent and one normal component. In these patients the incompetent component was dealt with adequately (i.e. a technically 'ideal' outcome). It was not possible to ascertain which of these possibilities was the most likely in retrospect, but some patients had very complex popliteal venous anatomy and the latter explanation is favoured. The reliability of duplex imaging is therefore open to doubt and a potential contributor to these results.

De Maeseneer and colleagues[8] demonstrated the reliability of colour-coded duplex examination in identifying the exact position of the SPJ to within 1 cm. They also showed that, in the case of recurrence, the position of the SPJ varied between 5 and 10 cm above the popliteal skin crease. This illustrates the importance of unrecognized anatomical variations (particularly high junctions) as a potential cause of technical failure of SPD and, therefore, recurrence. In this study four patients had unusually high incompetent junctions, together with an anatomically conventional and competent SPJ below. A fifth patient had incompetent junctions both at the conventional site and also several centimetres above the popliteal knee crease. In this group with a posterior thigh perforating vein, four of five patients had successful ligation of the incompetent vein. In a recent publication Caggiati[7] described a similar venous perforator occurring in 5 per cent of a healthy population. No patient with isolated Giacomeini vein incompetence was identified in the present study.

Three of ten patients with venous ulceration and four of nine with preulcerative skin changes were in the 'surgical failure' group; failure was less common in patients with uncomplicated varicose veins (four of 31), but the numbers were small. This difference may reflect the complexity of reflux in patients with chronic venous sufficiency. This was an open study and the lack of a consistent learning curve suggested that more comprehensive popliteal fossa dissection did not improve the results. Indeed, it may have contributed to popliteal vein injury in one case.

Given these relatively poor results (only 59 per cent of popliteal fossae were free of deep to superficial reflux after surgery) and the definite risks, there may be a case for avoiding SPD in patients with uncomplicated varices. Redo SPD for recurrence is likely to prove even less effective and more risky. In the present series, two of three patients who underwent redo surgery for persisting incompetent SPJ had complete failure of SPD; only one patient had a satisfactory duplex result.

SPD could be reserved for patients with complicated varicose veins, although opinion differs as to the contribution of isolated short saphenous disease to ulceration[9–11], and the present study suggests that this is the group in which technical failure is most likely. Perhaps patients with uncomplicated short saphenous varices should be offered repeated multiple avulsions or sclerotherapy, with no surgical attempt to correct deep to superficial reflux.

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Original article

Percutaneous radiofrequency thermoablation as an alternative to surgery for treatment of liver tumour recurrence after hepatectomy

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Background: Radiofrequency (RF) current, converted into heat through ion agitation and friction, can destroy liver tumours by means of coagulation necrosis. This study assessed whether percutaneous RF ablation is a useful and safe technique for the treatment of liver tumour recurrence after hepatectomy.

Methods: Forty-seven patients presenting with local recurrence after hepatectomy for malignant tumours (29 with colorectal secondaries) were treated with percutaneous RF ablation instead of repeat hepatectomy. RF thermal ablation was performed under image guidance for 12–15 min. This group represented 63 per cent of 75 patients treated with curative intent for liver recurrence in the same time interval. The other 28 patients underwent repeat hepatectomy.

Results: The mean(s.d.) number of liver metastases destroyed was 1·4(0·7) (range 1–3) and their diameter was 21(8) (range 9–35) mm. Twenty-six patients presented with liver recurrence at least once but up to three times after the initial RF application. Incomplete local RF treatment was observed in six of 47 patients. Fifteen patients developed extrahepatic recurrence. The mean(s.d.) interval between RF ablation and the last follow-up visit was 14·4(10·1) (range 5·5–40) months. One death and three major complications occurred. Survival rates at 1 and 2 years were 88 and 55 per cent respectively. A retrospective study of the authors' database over two similar consecutive periods showed that RF ablation increased the percentage of curative local treatments for liver recurrence after hepatectomy from 17 to 26 per cent and decreased the proportion of repeat hepatectomies from 100 to to 39 per cent.

Conclusion: Percutaneous RF treatment increases the number of patients eligible for curative treatment. It should be preferred to repeat hepatectomy when feasible and safe because it is less invasive. Repeat hepatectomy is indicated only when percutaneous RF ablation is contraindicated or fails.

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Introduction

Liver recurrence occurs in 50–70 per cent of patients following hepatectomy for primary or secondary tumours[1–4]. Until recently, when such recurrences were amenable to complete resection without major operative risk, repeat hepatectomy was the 'gold standard' treatment[5–8]. However, this surgical procedure is highly invasive and carries risks of postoperative mortality and morbidity[5–8]. For the past few years, the thermoablation technique[9–11] has been used both percutaneously[12,13] and intraoperatively[14,15] for the treatment of hepatic tumours. The percutaneous approach proved to be non-invasive and safe for moderately sized tumours (less than 3 cm in diameter)[12,13]. Usually liver recurrences are detected early, when they are still moderately sized, and are therefore eligible for radiofrequency (RF) treatment. Percutaneous RF ablation is simple to perform and less invasive than repeat hepatectomy. The aim of this study was to determine the value of percutaneous RF thermal ablation of liver recurrence after hepatectomy as an alternative to repeat hepatectomy.

Patients and methods

From January 1997 to January 2001, 75 patients who had previously undergone hepatectomy presented with either single or multiple resectable intrahepatic recurrent

Table 1 Initial liver resections

	No. of patients
Right hepatectomy	21
V + VI + VII + VIII	11
V + VI + VII + VIII + IV	8
V + VI + VII + VIII + I	2
Left hepatectomy	11
II + III + IV	6
II + III + IV + V	2
II + III + IV + I	2
II + III	1
Central hepatectomy	8
IV + V + VIII	3
V + VIII	2
IV + V	1
IV	2
Miscellaneous	7
Multiple metastasectomies	5
V + VI + VII	1
(II–III) + (VI–VII)	1

Mean(s.d.) number of lesions resected 3·8(2·6) (median 3·1 (range 1–10)). Extrahepatic lesions were resected in five patients

tumours. Since these lesions were limited in number, moderately sized and were not located close to a major vessel or bile duct, they were all considered to be resectable. RF treatment was preferred to repeat hepatectomy because it was less invasive, and was offered when there were fewer than five recurrent lesions of maximal diameter less than 3·5 cm, when lesions were accessible via a percutaneous approach and were situated more than 10 mm from the main bile ducts, and when no extrahepatic tumour was detected.

Among the 75 patients with potentially resectable liver recurrence, 47 (63 per cent) met the criteria for percutaneous RF treatment. Twenty-eight patients were not selected for RF thermoablation and underwent repeat hepatectomy because their liver lesions were either too close to the main bile ducts ($n = 9$), too numerous (more than four) ($n = 8$), or because an extrahepatic metastasis that required resection during the same operative procedure was present ($n = 11$).

The mean(s.d.) age of the 47 patients was 59·4(14·7) (range 13–85) years. The primary tumour was a colorectal cancer in 29 patients. The primary cancer in the remaining 18 patients was hepatocellular carcinoma (five patients), cholangiocarcinoma (two), neuroendocrine tumour (two), gastric carcinoma (two), soft tissue sarcoma (two), and one patient for each of the following primaries: melanoma, breast, kidney, thyroid and epithelioid haemangioendothelioma. Overall, eight patients had primary liver cancer and 39 patients had metastases from primary tumours at other sites.

Details of the initial hepatectomy procedures (before the development of recurrence) are reported in *Table 1*. Five of the 47 patients had an extrahepatic lesion at the time of the first hepatectomy, which was treated with a curative intent (R0 resection) during the same procedure. The median(s.d.) interval between the initial hepatectomy and RF ablation was 11·1(23·3) (range 2–44) months.

RF thermoablation was performed with a 200-W RF generator, either Radionics® (Burlington, Massachusetts, USA) with a cooled-tip electrode or Radiotherapeutics® (Mountain View, California, USA) with an expandable electrode. RF procedures were carried out under ultrasonographic guidance in 38 patients and under computed tomographic guidance (when the tumour was not seen ultrasonographically) in nine patients. The aim was to produce a necrotic sphere measuring at least 10 mm more than the tumour diameter. Details of the procedure have been reported previously[13,14]. RF ablation was performed under general anaesthesia with a one-night hospital stay in 31 patients and under local anaesthesia on an outpatient basis in 16 patients.

In treatment of the first liver recurrence, 65 tumours were treated in the 47 patients. The mean(s.d.) number of metastases destroyed was 1·4(0·7) (range 1–3) and the diameter was 21(8) (range 9–35) mm. Thirty-five patients had one tumour, seven patients had two, four patients had three and one patient had a total of four tumours.

Eighteen patients who presented with another liver recurrence following the first RF treatment underwent a second RF application. The mean(s.d.) interval between the first and second RF application was 6·1(2·5) (range 2–18) months. The details of these successive RF treatments are reported in *Table 2*. The total number of tumours destroyed in patients undergoing a second RF ablation treatment was 34 (mean number per patient 1·9) and the mean(s.d.) tumour size was 16(6) (range 10–28) mm. Four patients received RF treatment a third time for a third tumour recurrence in the liver (two lesions in each patient). In summary, a total of 107 recurrent liver tumours were treated with percutaneous RF ablation (65 at the first session, 34 at the second session and eight at the third session) in 47 patients.

Computed tomography (CT) and magnetic resonance imaging (MRI) were used for follow-up of patients every 2 months for 6 months and every 3 months for 2 years; CT and MRI were both performed on the same day[16]. No patient was lost to follow-up. The mean(s.d.) duration of follow-up between the initial RF treatment and the last follow-up visit was 14·4(10·1) (range 5·5–40) months.

Results

One death occurred in the series, attributed to portal thrombosis (mortality rate 2 per cent). It was the first death

Table 2 Outcome of percutaneous radiofrequency ablation for liver recurrence after hepatectomy

	RF1		RF2		RF3	
	Patients	Lesions	Patients	Lesions	Patients	Lesions
No recurrence	15	23	3	3	1	2
Liver recurrence						
New lesion treatable with RF	12	17	1	3	0	0
New lesion not treatable with RF	6	7	6	14	1	2
Local failure of RF	5	5	2	3	0	0
New lesion + local failure of RF	1	2	1	1	0	0
New lesion + extrahepatic recurrence	2	2	4	9	0	0
Extrahepatic recurrence	6	9	1	1	2	4
Total	47	65	18	34*	4	8†

*Including the seven lesions not completely destroyed with the first radiofrequency treatment (RF1); †including the four lesions not completely destroyed with the second treatment (RF2)

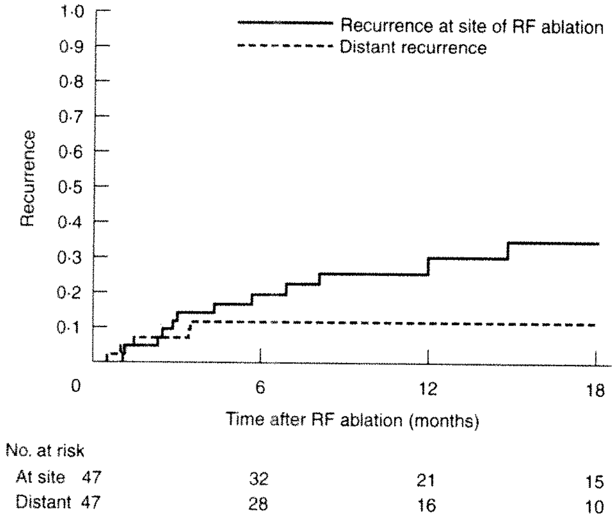


Fig. 1 Incidence of repeated liver tumour recurrence after treatment with percutaneous radiofrequency (RF) ablation, distinguishing local failure after RF treatment from new recurrence

observed in 285 RF procedures since 1997. Two bile abscesses requiring percutaneous drainage and one peritoneal haemorrhage (on the third postoperative day) treated by laparotomy occurred, giving a morbidity rate of 9 per cent among 47 patients, 6 per cent among 69 RF procedures and 4 per cent among 107 thermoablated lesions.

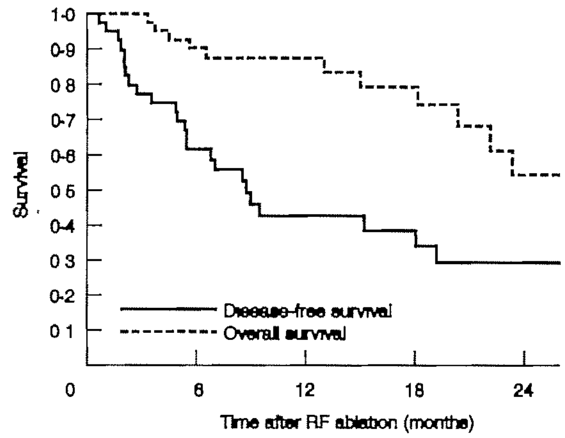
Following RF ablation of the first liver recurrence, 26 patients presented with further tumour in the liver, after a mean(s.d.) interval of 5·4(2·9) (median 5·5 (range 2–9)) months. Two of these patients also had an extrahepatic recurrence. Six of these 26 liver recurrences (9 per cent of the 65 thermoablated lesions) were at the site of the first RF application, indicating local failure of the procedure, and 18 were new hepatic lesions at other sites. The local failure was isolated in five patients and associated with new lesions in one patient. Nineteen patients (38 per cent) were still disease free at the time of writing (*Table 2*).

Percutaneous repeat RF ablation was performed in 18 of these 26 patients to treat new liver recurrence. RF treatment was contraindicated in eight patients, in six for technical reasons (of whom four patients had a repeat hepatectomy instead) and in two because of the presence of an extrahepatic lesion. Of the 18 patients, six developed a third liver recurrence after a mean(s.d.) interval of 3·4(3·4) (range 1·5–7) months.

The incidence of the two types of recurrence after RF ablation (at and outside the RF site) is reported in *Fig. 1*. An extrahepatic recurrence occurred in 15 patients after a mean delay of 6·8 months after the first RF ablation, in six patients associated with a new liver recurrence (*Table 2*).

After a median follow-up of 14·4 months, 34 patients are still alive: ten patients have recurrent liver tumour, six have extrahepatic recurrence (with or without liver recurrence) and 18 patients are disease free. One patient died with no evidence of disease progression. Overall and disease-free survival curves after RF ablation of recurrent liver tumour are shown in *Fig. 2*; 1- and 2-year survival rates were 88 and 55 per cent respectively.

For comparison of survival, this series was matched retrospectively with a series of 46 repeat hepatectomies performed previously in the same institution, which comprised almost the same number of metastatic colorectal cancers ($n = 28$) but included five neuroendocrine tumours[8]. The follow-up regimen was the same as that in the present series except that MRI was not used routinely. Survival rates at 1 and 2 years were 84 and 60 per cent respectively, similar to those described for RF ablation in



No at risk	0	6	12	18	24
Disease free	43	22	11	9	3
Overall	47	36	23	16	8

Fig. 2 Overall and disease-free survival curves after treatment of liver tumour recurrence with percutaneous radiofrequency (RF) ablation

the present article. The survival curves were similar for the two groups (hepatectomy *versus* RF ablation).

Discussion

Until now repeat hepatectomy has been considered the treatment of choice for recurrent liver tumours, yielding survival rates comparable with those of first hepatectomy[5–8]. Repeat hepatectomy has two advantages over a minimally invasive approach. First, the abdominal cavity can be explored and extrahepatic lesions (found in 10–20 per cent of patients) resected[7]. Second, complete resection of the recurrence is virtually guaranteed. However, there are major disadvantages to repeat hepatectomy: (1) a repeat laparotomy requires hospitalization, general anaesthesia, induces pain and involves high social and economic costs; (2) the related mortality rate is close to 2 per cent in specialized centres[5–8] and higher in non-specialized centres, and the postoperative morbidity rate is 25–30 per cent[5–8]; and (3) repeat hepatectomy is technically more difficult to perform than a first hepatectomy.

In contrast, percutaneous RF ablation presents major advantages over repeat hepatectomy. It is non-invasive, hospitalization is very short, pain is less severe, and associated mortality and morbidity rates are low. The RF mortality rate (2 per cent) has been overestimated in the present series. Considering that only one death has been observed in 285 RF procedures performed in the same institution, the true mortality rate is probably less than 1 per cent[17]. The social and economic impacts would be obvious if RF survival results were similar to those of repeat hepatectomy.

Although very appealing, this technique has three limitations. First, tumour destruction might be incomplete (six of 47 patients in this preliminary study). However, with close follow-up, residual disease can be detected early by MRI[16] and treated either with repeat RF ablation or repeat hepatectomy. Incomplete destruction occurred more frequently in large tumours (recurrences in this series mainly involved lesions greater than 30 mm in diameter) which necessitated more than one RF delivery.

The second limitation relates to the tumour localization. RF may be contraindicated (1) when the tumour is located too close to a main bile duct because of the risk of heat-induced stenosis, (2) if the lesion is at the hepatic dome owing to a risk of right atrium injury or (3) if the liver tumour is adjacent to the right colon to avoid necrotic colon injury.

The third drawback is that the percutaneous approach does not allow either adequate inspection of the abdominal cavity or ultrasonographic liver exploration (which detected an additional lesion in 6 per cent of patients)[18].

Finally, two questions need to be addressed regarding percutaneous RF application for treatment of recurrent liver tumour following a first hepatectomy. First, will RF ablation be able to replace repeat hepatectomy? The present study suggests that RF treatment could replace repeat hepatectomy when the site, number and size of the lesions allow it to be used safely. Comparison of the present data with the survival results obtained in the same institution in 1993 after repeat hepatectomy for liver recurrence in 46 patients[8] revealed similar 1- and 2-year survival rates after RF ablation (88 and 55 per cent respectively) and repeat hepatectomy (84 and 60 per cent respectively). However, a possible selection bias resulting from comparison of two non-randomized groups of patients precludes any definitive conclusion to be drawn. Nevertheless, similar results have been described in other published series: survival rates at 1 and 2 years after repeat hepatectomy were 87 and 52 per cent respectively in 191 patients reviewed by Neeleman and Andersson[6], very similar to those in 143 patients reported by the Association Française de Chirurgie[3], and those in 170 patients reported in the Repeat Hepatic Resection Registry[7], which all included the authors' 1993 series.

Second, will RF allow treatment with curative intent in more patients than repeat hepatectomy? The answer is clearly yes considering that, since it is minimally invasive, RF is proposed earlier and to a greater number of patients than classical repeat hepatectomy. A retrospective analysis of the surgical practice over two similar consecutive periods in the same centre shows that the use of RF thermal ablation has increased the incidence of curative local treatment for liver tumour recurrence from 17 to 26 per cent and

Table 3 Evolution in the treatment of liver recurrence after hepatectomy during two consecutive periods at Institut Gustave Roussy

	August 1995 to March 1997	April 1997 to November 1999
Duration (months)	20	20
No. of hepatectomies	108	157
Local treatments of liver recurrence	18 (17)	41 (26)
No. of repeat hepatectomies	18 (100)	16 (39)
No. of radiofrequency treatments	0 (0)	25 (61)

Values in parentheses are percentages

decreased the incidence of repeat hepatectomy from 100 to 39 per cent (*Table 3*).

In conclusion, while awaiting the results of a randomized trial (including an analysis of quality of life and costs) comparing repeat hepatectomy with percutaneous RF application for the treatment of tumour recurrence after hepatectomy, both treatment modalities are acceptable. However, since RF ablation is much less invasive than repeat hepatectomy, it may increase the number of patients eligible for curative treatment. In this context, patient recruitment in a randomized study (needing several hundreds of patients to reach statistical significance) will probably be difficult. According to the data already available, RF treatment is safe and effective. It should become the preferred treatment for liver recurrence after hepatectomy, the only remaining indications for repeat hepatectomy being technical contraindications to percutaneous RF ablation and local failure of this technique.

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Original article

Percutaneous fine-needle aspiration cytology in the diagnosis and management of liver tumours

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Background: The aim of the study was to investigate the value of percutaneous fine-needle aspiration cytology (FNAC) in the diagnosis and management of liver tumours.

Methods: FNAC followed by histopathological examination was carried out in 216 patients with suspected liver tumours. The final diagnosis was primary liver cancer in 106, colorectal metastases in 51, non-colorectal metastases in 46, benign tumour in nine and no tumour in four patients.

Results: Cytology resulted in correct classification of the lesion as benign or malignant in 87 per cent of patients, correct discrimination between primary and secondary malignancy in half of the patients, and a correct diagnosis of tumour type in one-third of patients. The tumour was erroneously classified as benign or malignant in 22 patients (11 per cent) and four patients (2 per cent) respectively. When FNAC showed malignancy, the predictive value was 98 per cent, whereas the predictive value was 27 per cent when it did not. FNAC guided investigations and treatment in one-quarter of patients. Implantation metastases were recorded in seven patients (3 per cent), including five (10 per cent) of 51 patients with colorectal liver metastases, and caused major local problems and death in four patients.

Conclusion: FNAC was valuable in about a quarter of patients with liver tumour. The risks of implantation metastases and a false-negative finding do not justify its use in candidates for curative therapy of liver tumours.

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Introduction

Percutaneous biopsy with a fine needle (diameter less than 1 mm) has been used extensively since the beginning of the 1970s. It is an established procedure for obtaining specimens for cytological diagnosis of liver tumours, with a sensitivity for detection of liver malignancy of between 69 and 97 per cent[1–13]. However, there are no reports of its influence on treatment outcome.

Major morbidity is unusual (0·05–0·18 per cent), and the reported mortality rate varies from 0·01 to 0·03 per cent after fine-needle aspiration cytology (FNAC) of various organs[14–17]. Although experimental data indicate a considerable risk of tumour seeding of the needle tract[18,19], the risk of implantation metastases has been calculated to be less than 0·01 per cent in patients[14,15,17]. During recent years, however, several case reports on implantation metastases after FNAC of liver tumours have been published[20–28].

The purpose of the present study was to analyse the diagnostic value and clinical impact of FNAC of liver tumours.

Patients and methods

This was a retrospective study comprising all 216 patients (112 men, 104 women; median age 61 (range 23–86) years) undergoing FNAC and histopathological examination of a suspected liver tumour from 1973 to 1997. At the time of review, 26 patients were alive (at a median follow-up of 48 (range 12–261) months), 188 patients were dead and two patients were lost to follow-up.

The indications for FNAC were suspected hepatic metastasis from a known ($n = 65$), and usually resected ($n = 61$), extrahepatic malignancy, liver tumour of unclear origin or type ($n = 148$), and suspected recurrence of liver tumour ($n = 3$).

Fine-needle aspiration was carried out using 20- or 22-G (outer diameter 0·7–0·9 mm) needles. The aspirations were performed by radiologists under fluoroscopic, computed topographic or ultrasonographic guidance in 135 patients. Cytologists performed aspirations on a palpable tumour in 55 patients and blindly in 26 patients. FNAC was repeated on a second or third occasion in 36 and nine patients

Table 1 Diagnostic outcome of fine-needle aspiration cytology of liver tumours

		Cytological diagnosis					
Histopathological diagnosis	*n*	Correct benign/ malignant	Correct primary/ secondary	Correct tumour type	Incorrect benign diagnosis	Incorrect malignant diagnosis	No representative material
All	216	178 (87)	94 (49)	73 (36)	22 (11)	4 (2)	12
Primary malignancy	106	90 (89)	38 (38)	22 (22)	11 (11)		5
Hepatocellular carcinoma	56	53 (96)	32 (58)	20 (36)	2 (4)		1
Hilar cholangiocarcinoma*	13	6 (60)	1 (10)	1 (10)	4 (40)		3
Cholangiocellular carcinoma	17	16 (94)	3 (18)	1 (6)	1 (6)		
Gallbladder carcinoma	9	7 (78)			2 (22)		
Other	11	8 (80)	2 (20)		2 (20)		1
Secondary malignancy	97	80 (88)	56 (62)	49 (54)	11 (12)		6
Colorectal	51	40 (89)	35 (78)	29 (64)	5 (11)		6
Non-colorectal	46	40 (87)	21 (46)	20 (43)	6 (13)		
Benign tumour	9	5 (63)		2 (25)		3 (38)	1
Focal nodular hyperplasia	4	3 (100)					1
Haemangioma	2					2 (100)	
Hepatocellular adenoma	2	2 (100)		2 (100)			
Hepatobiliary adenoma	1					1 (100)	
No tumour	4	3 (75)				1 (25)	
Cirrhosis	2	2 (100)					
Abscess	1	1 (100)					
Normal liver	1					1 (100)	

Values in parentheses are percentages based on specimens containing representative material. *Klatskin tumour; all had hepatic overgrowth

respectively. The samples were fixed with ethanol and stained with haematoxylin and eosin, or fixed with air and stained with May–Gruenwald–Giemsa, or both.

The histopathological specimen was obtained by laparotomy in 140 patients, laparoscopy in three, percutaneous core biopsy (coarse needle) in five and autopsy in 68 patients. The final histopathological diagnoses are shown in *Table 1*.

Cytological and histopathological re-examination was carried out whenever there was a false-positive or false-negative cytological diagnosis of malignancy. To obtain a blinded re-examination, specimens with an incorrect diagnosis were mixed with the same number of specimens with a correct diagnosis. In three patients, the cytological specimens were damaged and could not be re-evaluated.

All biopsies were classified as showing benign changes, suspicion of malignancy, malignancy or no representative material (i.e. insufficient or uninterpretable material). Excluding cytological specimens with no representative material, the ability of FNAC to detect or exclude malignant disease was estimated by calculating the percentage of correct classifications, sensitivity, specificity and predictive values. In these calculations, suspected malignancy was regarded as malignancy. The diagnostic outcome of FNAC was further estimated by its ability to distinguish between primary and secondary tumour and its ability to classify tumour type correctly, i.e. subgroup of primary liver tumour or origin of metastatic tumour.

Results

Cytology correctly classified the lesion as benign or malignant in 178 instances (87 per cent), including ten patients (5 per cent) in whom the findings were described as suspected malignancy (*Table 1*). All patients with suspected malignancy on FNAC had malignant disease. A correct distinction between primary and secondary malignancy was made in 94 cases (49 per cent of patients with malignancy) and a correct diagnosis of tumour type was established in 73 patients (36 per cent).

The diagnostic outcome is summarized in *Table 2*. FNAC was able to detect or exclude malignant disease with a sensitivity of 89 per cent, specificity 67 per cent, positive predictive value 98 per cent and negative predictive value 27 per cent. These figures were similar for primary and secondary tumours. After re-examination, the sensitivity was 91 per cent, specificity 100 per cent, positive predictive value 100 per cent and negative predictive value 40 per cent. It appears that the sensitivity was higher after 1982 than before (93–97 *versus* 79–86 per cent), and that the ability of FNAC to classify tumour type correctly improved after 1982.

Table 2 Diagnostic outcome and implantation metastases at different time intervals

	1973–1997	1973–1977	1978–1982	1983–1987	1988–1992	1993–1997
No. of patients	216	46	64	36	34	36
Diagnostic outcome (%)						
Benign/malignant disease						
Sensitivity	89	79	86	97	93	93
Specificity	67	100	100	100	67	25
Positive predictive value	98	100	100	100	96	90
Negative predictive value	27	10	27	50	50	33
Primary/secondary tumour	49	38	47	48	69	50
Tumour type	36	16	28	47	59	41
No representative material (%)	6	7	5	6	6	6
No. of implantation metastases	7 (3)	2 (4)	0	1 (3)	4 (12)	0 (0)

Values in parentheses are percentages

The diagnostic yield was similar after blind puncture, image-guided puncture and that performed on a palpable tumour.

Misclassifications

The lesion was erroneously classified as benign in 22 patients (11 per cent) (*Table 1*). Ultrasonographic guidance was used in 17 of these patients, and aspirations were repeated once in four patients and twice in one patient. The false-negative rate was similar for different diagnoses (4–22 per cent), with the exception of hilar cholangiocarcinoma (Klatskin tumour) with intrahepatic growth, for which it was 40 per cent. Median size of the tumour was 8 cm in patients with a false-negative FNAC.

Re-examination of specimens incorrectly judged as benign changed the diagnosis to malignancy in two of the 19 cases that could be re-evaluated (initial interpretation error). Thirteen specimens were again considered to contain only normal liver or duct cells, whereas four were considered to contain no representative material. All re-examinations of specimens with correct primary diagnoses (used to blind the re-examination procedure) gave the same result as the primary assessment.

Four patients (2 per cent) with benign tumours (haemangioma (n = 2), hepatobiliary adenoma (n = 1)) or normal liver (n = 1) had a malignant diagnosis at FNAC. At re-examination two cytologies were judged to be inconclusive, whereas two specimens were regarded as benign (final diagnoses were haemangioma and normal liver).

Effect on patient management

Considering the information available when FNAC was performed, cytology gave new correct information in 76 patients (35 per cent). It confirmed a previously suspected diagnosis in 90 patients (42 per cent), was inconclusive in 22 patients (including ten with suspected malignancy) (10 per cent), and incorrect in 28 patients (13 per cent). The latter figure includes two patients with primary liver cancer in whom cytology classified the lesions as metastases from extrahepatic malignancy, resulting in further unnecessary investigations.

The cytological diagnosis guided further investigations and treatment in 56 patients (26 per cent) and had no or equivocal consequences in 150 patients (69 per cent). The latter figure includes 20 patients in whom cytology gave new and correct information but in whom subsequent imaging, ordered before execution of FNAC, gave the same information. In the remaining ten patients (5 per cent) cytology resulted in repeated aspiration without a correct diagnosis (n = 5), other unnecessary examinations (n = 2), delay in making the correct diagnosis (n = 3) and unnecessary surgery (n = 2).

Complications

The main complication of FNAC was the occurrence of implantation metastases in seven patients (3 per cent), including five (10 per cent) of 51 patients with colorectal liver cancer (*Table 3*). A 20- or 22-G needle was used, and aspiration was guided by imaging in five patients, performed by a cytologist on a palpable tumour in one patient and blindly in one patient. The aspiration was repeated on a second occasion in two patients.

Four patients had implantation metastases involving the thoracic wall, intercostal muscles, pleura, right lung, diaphragm and peritoneum; and three patients had metastases in the skin and subcutaneous tissue of the abdominal wall. The interval between FNAC and detection of implantation metastases was 2–49 (median 4) months. Three of seven patients with implantation metastases had

Table 3 Patients with implantation metastases

				Implantation metastases						
Patient	Type of liver tumour	Treatment	Other recurrences*	Time to detection (months)†	Localization	Resection‡	Survival after resection (months)	Recurrence	Local problems	Impact on survival
1	Colorectal	Resection		7	Thoracic wall	Not radical	5	Yes	Major	Yes
2	Colorectal	Resection		12	Abdominal wall	Radical	26	Yes	Major	Yes
3	Colorectal	Resection	Lung	49	Thoracic wall	Radical	26	Yes	Major	Yes
4	Colorectal	Dearterialization		3	Thoracic wall	No	—	—	Major	Yes
5	Colorectal	Dearterialization	Lung	4	Abdominal wall	Radical	4	No	Minor	No
6	Metastases from gallbladder carcinoma	Dearterialization	Brain	2	Abdominal wall	Radical	2	No	Minor	No
7	Hepatocellular carcinoma	Resection (not radical)		2	Thoracic wall	No	—	—	Minor	No

*All were detected after detection of implantation metastases. †Interval between fine-needle aspiration cytology and detection. ‡Intraoperative judgement of radical resection

undergone radical resection of the liver tumour(s); implantation disease was the only demonstrable recurrence in one of them (patient 1, *Table 3*) and the initial and major recurrence in two (patients 2 and 3, *Table 3*). Implantation metastases preceded other recurrences in all but one patient (patient 7, *Table 3*).

Implantation metastases caused major local problems and were fatal in four patients. Resection of implantation metastases was performed in five patients and was considered locally radical in four. Two of these patients had undergone dearterialization and lived for only 2–4 months after resection of implantation metastases, whereas there was recurrence at the implantation site in the other two.

Discussion

With respect to its ability to establish the presence of malignancy, the sensitivity of FNAC was 89 per cent, which compares well with previous studies. The specificity was only 67 per cent, which is lower than reported previously; most authors report figures higher than 95 per cent[1–13]. The low specificity in this study may largely be explained by the small number of benign lesions.

The diagnostic value of a test depends on its sensitivity and specificity, and the distribution of patients with and without disease in the study population. Predictive values are relevant in the clinical situation. The high number of patients with malignancy ($n = 203$) and the low number of patients without malignancy ($n = 13$) reflected referral policy and the fact that most solid liver tumours are malignant. As a result, the predictive value of a negative test was low (27 per cent) and that of a positive test was high (98 per cent). High sensitivity (or predictive value of a negative test) is important if the therapy is potentially curative. High specificity (or predictive value of a positive test) prevents unnecessary suffering.

Only normal liver or ductal cells were found in most of the false-negative aspirates. The comparatively low false-negative rate during the past 15 years might reflect improved guidance and puncture techniques (*Table 2*). Sensitivity was relatively low for Klatskin tumours (with intrahepatic tumour growth) for which FNAC was positive in only 60 per cent of cases (*Table 1*). Klatskin tumours at the liver hilum are deeply located, in addition to being firm and fibrous, which might explain the low ability of FNAC to establish a correct diagnosis.

Although the risk of false-positive cytology is believed to be negligible, a rate of 1–4 per cent has been reported[1–3,7,8,12]. Inflammatory conditions with regenerative activity of the liver may cause variation in nuclear size, enlarged nucleoli, multinucleated cells and the presence of mitoses[1]. Such changes may be misinterpreted as malignant on cytological examination, and this was probably the case in one of the present patients, who had had an episode of bile stone cholangitis at the time of FNAC.

An incorrect diagnosis of benign or malignant disease was made in 26 patients. As judged from the re-examinations, misclassification was due to erroneous interpretation of the cytological findings in four patients and failure to obtain representative material in 19 patients.

In clinical practice the value of FNAC depends on the results of examinations already performed. For instance, cytology of a liver tumour demonstrated on imaging can at best confirm liver metastasis in a patient with a history of colorectal cancer and a raised serum carcinoembryonic antigen level. Since the predictive value of FNAC showing no malignancy is about 30 per cent, a normal cytological examination in this situation would not preclude malignancy. On the other hand, a positive finding on cytology will be of clinical importance in a patient with unclear findings on imaging and normal levels of tumour markers. This was the case in 26 per cent of the patients in this study.

Based on a study of 11 700 patients, Livraghi *et al.*[14] (own series and literature review) estimated that the rate of major complications after fine-needle abdominal biopsies was 0·05 per cent, with a mortality rate of less than 0·01 per cent. At that time (1983), only two cases of needle-tract seeding after FNAC had been described in the literature. Tumour cell seeding of the needle tract after FNAC has been reported to occur with a frequency of 65–90 per cent in different experimental situations[18,19]. Since hardly any clinical cases of implantation metastases after FNAC were reported during the 1970s and 1980s, it was believed that tumour cells in the needle tract were destroyed by the host immune response, or by some other mechanism[15]. However, the belief that the risk of tumour seeding after FNAC of the liver is negligible has been questioned by case reports published in recent years[20–28].

Several factors contribute to the difficulty in making a reliable estimation of the risk of needle-tract seeding after FNAC. Patients with liver malignancy are only rarely cured, and implantation metastases can easily be overlooked in the presence of progressive disease. Untreated patients with advanced liver tumour usually have a short survival time, and the time for development of detectable implantation metastases will therefore be short; the median lead time was 4 months in the present study. The risk of implantation metastases after FNAC is probably best estimated in patients with radically resected liver tumours and prolonged survival. This may explain the observed frequency of implantation metastases in the present study; four of seven patients with implantation metastases had undergone liver resection with curative intent.

In organs in which the needle tract is excised together with the tumour, for instance in breast cancer, FNAC is safe with respect to implantation metastases. However, in liver, pancreas and other organs in which the needle tract will be left behind at tumour resection, the consequences of tumour seeding may be ominous. Frequently, fine-needle aspiration of liver tumour must be performed via a transthoracic approach, which constitutes a special problem. Implantation metastasis of the thoracic wall, affecting the peritoneum, diaphragm, pleura, lung, intercostal muscles or ribs, is difficult or impossible to resect.

In conclusion, FNAC can distinguish between benign and malignant liver tumours in 87 per cent of cases. The risk of implantation metastases and false-negative findings means that FNAC should be avoided in candidates for curative hepatic surgery such as resection or transplantation.

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Original article

The Surgical Risk Scale as an improved tool for risk-adjusted analysis in comparative surgical audit

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Background: Comparative surgical audit is becoming increasingly important although it is fraught with difficulties due to risk-adjusted analysis. Methods have been proposed to solve this problem and allow meaningful comparison of patient outcome. None has been described without faults, making such comparison flawed or overtly complicated. An alternative risk scoring system incorporating the Confidential Enquiry into Perioperative Deaths (CEPOD) grade, the American Society of Anesthesiologists (ASA) grade and the British United Provident Association (BUPA) operative grade was formulated and assessed.

Methods: This was a prospective audit of 4308 patients admitted under the care of three surgeons between May 1997 and October 1999, creating an initial data set of 3144 procedures with 134 deaths. Each procedure was allocated a score on the basis of the CEPOD, BUPA and ASA grade. The Surgical Risk Scale (SRS) was devised by adding together the values of the three variables, which generated a scale ranging from 3 to 14. Multivariate logistic regression analysis involving the three variables and univariate analysis of the SRS score were undertaken. Receiver–operator characteristic and calibration curves were formulated. This process was validated on another data set (2780 patients) derived from all admissions to the same surgeons between November 1999 and December 2000.

Results: Univariate logistic analysis of the SRS score revealed it to be significantly predictive of death ($\beta = 0.84$, $P < 0.001$); it did not overpredict mortality for low-risk procedures.

Conclusion: The SRS is easy to use, formulate and interpret, and provides an accurate prediction of death in general surgical patients across the entire risk spectrum.

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Introduction

Comparative surgical audit is becoming increasingly important within the surgical profession, and to healthcare managers, the government and the general public. It should allow comparison of morbidity and mortality rates between institutions, teams and individual surgeons. League tables with comparisons between different surgical units and individual surgeons are likely to be adopted.

However, data collection methods have yet to be standardized and meaningful comparison is not yet possible. Simple collection of numbers alone is insufficient to reflect the quality of care because, to compare morbidity and mortality directly, the original populations should be identical. True comparisons can be made only if risk adjustment is used to allow for the differences in case mix between units and surgeons.

A number of methods has been proposed for standardizing patient data to permit direct comparison of patient outcome irrespective of differences in population[1–4]. Foremost among these in the UK is the Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM), which is currently the most tested system for assessing outcomes by risk-adjusted analysis[5–9].

While POSSUM has performed favourably, it has its limitations. Most notably, it and a subsequent modification (Portsmouth (P) POSSUM) consistently overestimate the mortality rate for low-risk procedures[2,6,7,9]. The formula for establishing a POSSUM score is complex and the relative performance of POSSUM and P-POSSUM depends on the method of analysis[2,6,8]. The operative variables collected to calculate POSSUM might contrive to obscure poor surgical standards. A high total blood loss or

Table 1 Scoring system

	Description	Score
CEPOD		
Elective	Routine booked non-urgent case, e.g. varicose veins or hernia	1
Scheduled	Booked admission, e.g. cancer of the colon or AAA	2
Urgent	Cases requiring treatment within 24–48 h of admission, e.g. obstructed colon	3
Emergency	Cases requiring immediate treatment, e.g. ruptured AAA	4
BUPA		
Minor	Removal of sebaceous cyst, skin lesions, oesophagogastric duodenoscopy	1
Intermediate	Unilateral varicose veins, unilateral hernia repair, colonoscopy	2
Major	Appendicectomy, open cholecystectomy	3
Major plus	Gastrectomy, any colectomy, laparoscopic cholecystectomy	4
Complex major	Carotid endarterectomy, AAA repair, limb salvage, anterior resection, oesophagectomy	5
ASA		
I	No systemic disease	1
II	Mild systemic disease	2
III	Systemic disease affecting activity	3
IV	Serious disease but not moribund	4
V	Moribund, not expected to survive	5

The Surgical Risk Scale score was calculated for each procedure by adding the Confidential Enquiry into Perioperative Deaths (CEPOD), American Society of Anesthesiologists (ASA) and British United Provident Association (BUPA) scores. AAA, abdominal aortic aneurysm

the need for multiple procedures would automatically give a high POSSUM score and a high expected mortality or morbidity rate. However, these might result from poor surgical technique rather than from the intrinsic difficulty of the case. With regard to these variables, as well as to the presence of malignancy (which is also incorporated into the operative severity score), there is concern that these data are not appropriate or relevant outside general and gastrointestinal surgery. Other areas of specialization, such as vascular surgery and urology, will formulate their own POSSUM scoring systems, thus reducing its overall applicability and ability to provide meaningful comparison across the range of surgical specialties.

In response to these concerns the authors considered whether an alternative system, based on easily collected clinical data independent of the surgeon, might provide an accurate prediction of mortality. Clinical experience suggests that the mode of presentation, co-morbidity and the magnitude of the surgical procedure generally predict patient outcome. An alternative risk scoring system incorporating the Confidential Enquiry into Perioperative Deaths (CEPOD) grade, the American Society of Anesthesiologists (ASA) grade and the British United Provident Association (BUPA) operative grade was formulated and assessed.

Patients and methods

All patients admitted under the care of three surgeons between May 1997 and October 1999 were assessed. Patients transferred to the care of the firm while an inpatient and those whose care was on a shared basis with another firm were included. Each procedure was prospectively allocated a CEPOD category by the admitting physician, a BUPA category by the surgeon and ASA grade by the anaesthetist. Completeness of data collection and accuracy of grading and outcome were ensured at a weekly meeting of the surgical firm at which all patients discharged during the previous week were discussed. Hospital inpatient death was used as the outcome measure. All data were entered into a computerized database on a weekly basis and no reliance was made upon the patient administrative system run by the hospital.

Some 4308 patients were admitted during this period, of which 1351 (31 per cent) did not have an operation and were therefore excluded from further analysis. A total of 2957 patients had an operation (154 had more than one operation during the admission), of whom 96 died during their stay in hospital (in-hospital mortality rate 0·03 per cent). Ten operative records did not contain the full data set required (none associated with death) and were therefore excluded. A total of 3144 procedures were available for analysis, of which 134 were associated with a negative outcome (i.e. death).

The validation data set was derived from all admissions to the same three surgeons admitted between November 1999 and December 2000. Some 2780 patients were admitted during this period, of which 834 (30 per cent) did not have an operative procedure and were therefore excluded from further analysis. Of 1946 patients who had an operation (57 had more than one operation during the admission), 47 patients died during their stay in hospital (in-hospital

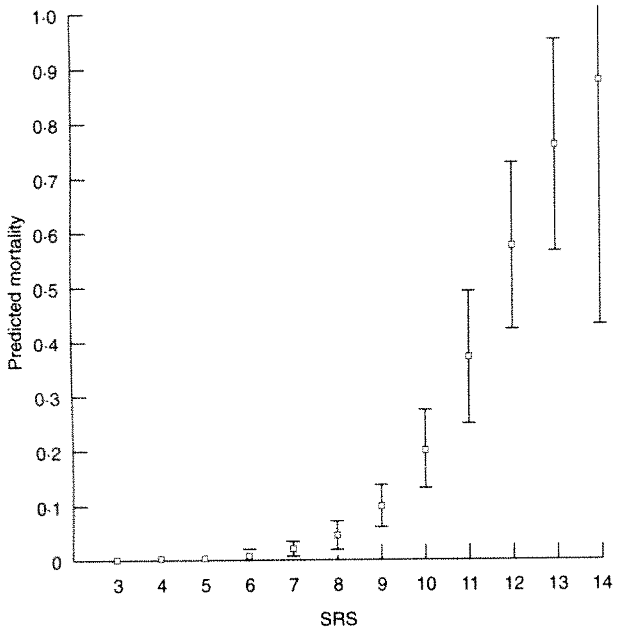


Fig. 1 Predicted operative mortality (95 per cent confidence intervals). SRS, Surgical Risk Scale

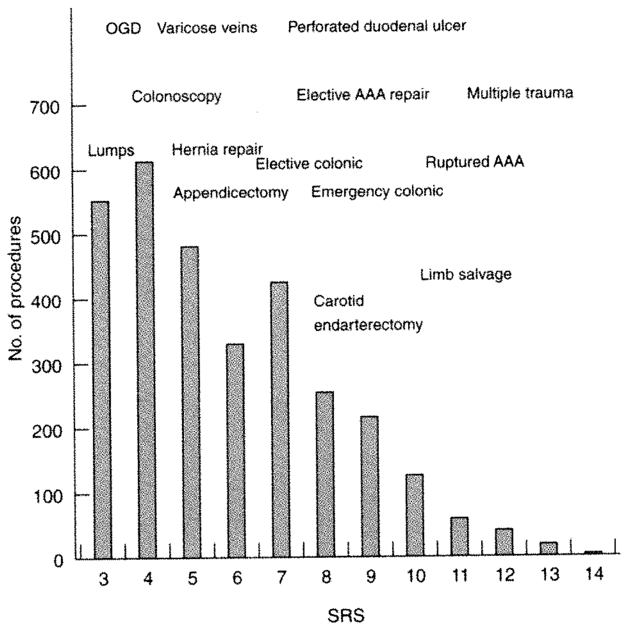


Fig. 2 Distribution of procedures undertaken (total 3144) with a schematic representation of where on the Surgical Risk Scale (SRS) the scores for various general surgical procedures lie. AAA, abdominal aortic aneurysm; OGD, oesophagogastric duodenoscopy

mortality rate 0·02 per cent). Seven operative records did not contain the full data set required (none associated with death) and were therefore excluded. A total of 2024 procedures were available for analysis, of which 62 were associated with a negative outcome (i.e. death).

The CEPOD and BUPA categories were allocated a linear ordinal scale (*Table 1*) and a multivariate logistic regression analysis involving three variables (CEPOD, BUPA and ASA grade) was undertaken. The logistic regression equation generated determined the association of death with the three variables, taking into account the relationship between the variables. A simple risk scale was designed (Surgical Risk Scale (SRS)) by adding together the values of the three variables (*Table 1*). This generated a scale ranging from 3 to a maximum of 14 against which a further univariate analysis was undertaken.

Validation involved testing the SRS scoring system on another data set. Predictive ability and goodness of fit were assessed using classification tables for 10, 50 and 90 per cent decision criteria, receiver–operator characteristic (ROC) curves and calibration curves[10–12]. Data analysis was undertaken in SPSS (SPSS, Chicago, Illinois, USA).

Results

Multivariate logistic regression analysis for the three scores (CEPOD, BUPA and ASA) revealed that each was independently significantly predictive of death (CEPOD $\beta = 0{\cdot}57$, $P < 0{\cdot}001$; BUPA $\beta = 0{\cdot}37$, $P < 0{\cdot}001$; ASA $\beta = 1{\cdot}68$, $P < 0{\cdot}001$; constant $= -9{\cdot}84$).

Univariate logistic analysis of the SRS score revealed it to be significantly predictive of death ($\beta = 0{\cdot}84$, $P < 0{\cdot}001$, constant $= -9{\cdot}81$). *Fig. 1* is a visual representation of the predicted mortality rate at each point on the SRS score. *Fig. 2* shows, for the initial data set, the number of procedures undertaken at each of the SRS scores with the text indicating broadly where a number of common general surgical procedures lie.

Goodness of fit of the Surgical Risk Scale model

Table 2 is the classification table for the decision threshold of 50 per cent. At one extreme, 10 per cent decision criteria gave a high sensitivity (62 per cent) but a reduced specificity (95 per cent). At the other extreme, with 80 per cent decision criteria the high specificity (100 per cent) was accompanied by a low sensitivity (2 per cent).

The calibration curves in *Fig. 3* show how the observed and predicted risk of death increase in proportion to one another. The diagonal line represents the point at which the observed to predicted ratio is 1·0 for each category of

Table 2 Classification table for Surgical Risk Scale variable analysis with decision criteria of 50 per cent

	Predicted		
	Alive	Dead	Total
Observed			
Alive	2983	27	3010
Dead	100	34	134
Total	3083	61	3144

Sensitivity 25 per cent, specificity 99 per cent and total correct classification (accuracy) 96 per cent

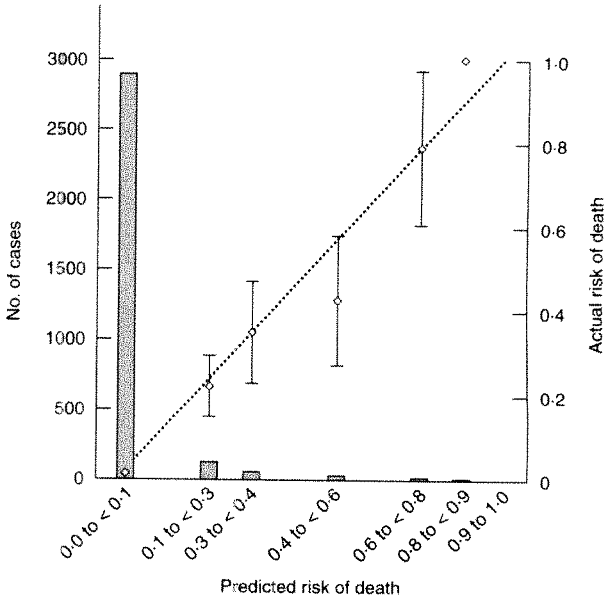
predicted death. The 95 per cent confidence intervals for the observed mortality rate at each level of predicted mortality encompass this line.

Fig. 4 shows the ROC curves for the initial data set; the area under the curve was 0·93. The Hosmer–Lemeshow test indicated that the model fits the data well ($\chi^2 = 3{\cdot}31$, 5 d.f., $P = 0{\cdot}65$). The ROC curve for the validation data set is also included for comparison.

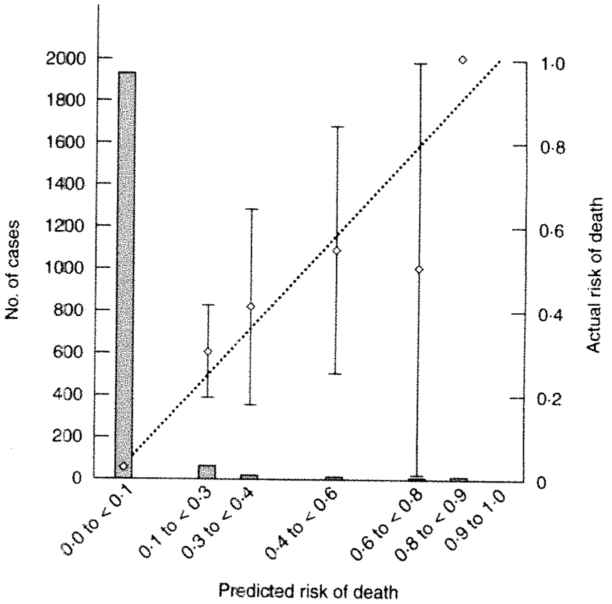
Discussion

When formulating a risk assessment scoring system for comparative surgical audit it is important that, while accurate, it should be quick and easy to use at the bedside. The variables collected should be small in number, be available for every patient undergoing any operation, be in common use across the healthcare system and leave little room for observer bias. The score should then be easy to calculate from the data collected. Furthermore, it should be possible to integrate the scoring system into pre-existing audit programmes with a minimum of disruption.

All three elements of the SRS fulfil these criteria. They are used commonly throughout the UK and the variables on which they are based are recorded for every patient undergoing surgery. No special investigations are needed. Only 17 (0·003 per cent) of the 4903 patients in this study could not be allocated an SRS score because there was insufficient information recorded in the case notes. A score can be calculated at the bedside and there are no complicated tables or calculations to make. The potential for interobserver error is small and it is difficult to inflate the score. The ASA grade alone leaves room for a small degree of subjective error, but it has the advantage of simplicity, of universal use and is known to be an effective risk indicator when used alone[13] or in combination with other parameters[3,14]. While there are concerns about the technical accuracy of the BUPA operative score with regard to some procedures, there is no room for error in interpretation and there is general agreement as to its applicability such that it is already in common use.



a Initial data set



b Vaidation data set

Fig. 3 Calibration curves (symbols with 95 per cent confidence intervals) for mortality for **a** initial data set and **b** validation data set. Bars indicate the number of patients in each predicted risk group. The diagonal line represents an observed : expected ratio of 1·00

This large prospective study has shown that the SRS is an effective tool for predicting death. It is at least as accurate as other scoring systems[1–4] and is less complicated. The

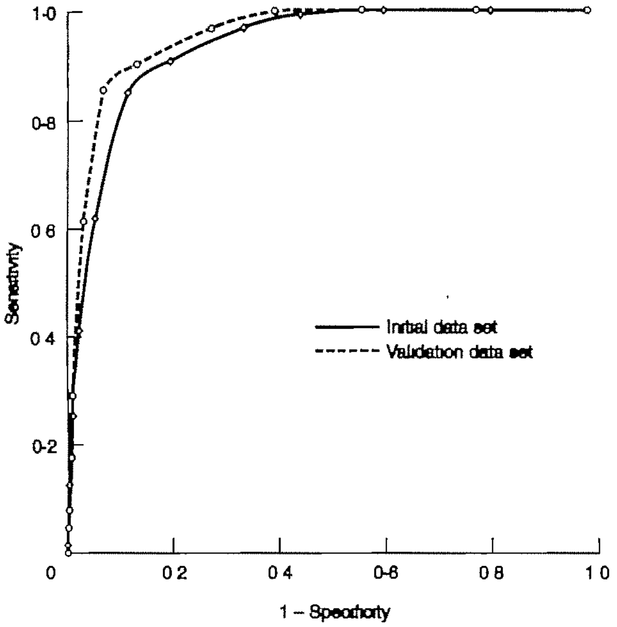


Fig. 4 Receiver–operator characteristic curves for mortality. Area under the curve was 0·93 for the initial data set and 0·95 for the validation data set

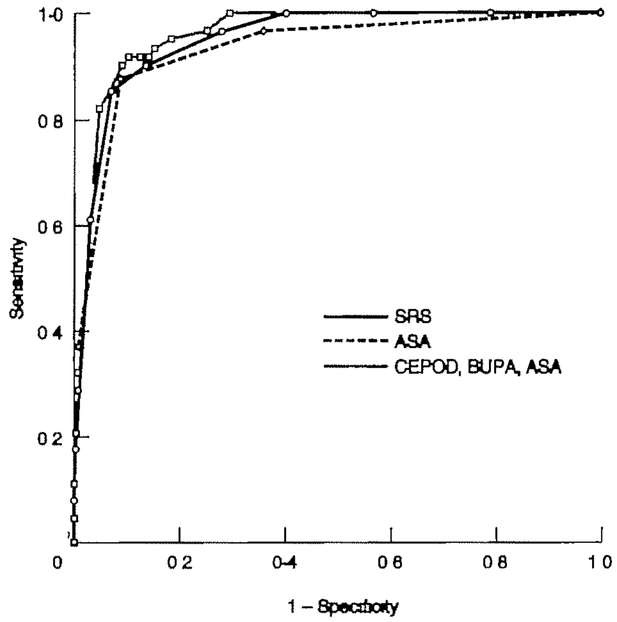


Fig. 5 Receiver–operator characteristic curves for mortality in the validation data set for the composite Surgical Risk Scale (SRS) score (univariate analysis), the American Society of Anesthesiologists (ASA) score alone, and the Confidential Enquiry into Perioperative Deaths (CEPOD), British United Provident Association (BUPA) and ASA scores combined (multivariate analysis). Areas under the curve (95 per cent confidence interval) were 0·95 (0·93 to 0·97), 0·93 (0·90 to 0·97) and 0·96 (0·95 to 0·98) respectively

goodness of fit is demonstrated graphically by the ROC curves (*Fig. 4*), for which the area under the curve is a measure of accuracy. For the SRS this was 0·95, which compares favourably with that for POSSUM at 0·84[4]. However, predicted mortality using the CEPOD, ASA and BUPA classifications in the multivariate model is slightly more accurate, and that for ASA alone almost as accurate (*Fig. 5*). The 'cost' in loss of predictive power incurred by using a simple pragmatic score (SRS) is minimal compared with the complexities of multivariate regression equations. An ASA score alone is even simpler to use but its very subjective nature, in the absence of 'operative' and 'mode of presentation' components, increases its susceptibility to variation (*Fig. 5*).

The vast majority of general surgical procedures undertaken fall into the low-risk category. The absolute minimum risk of mortality is 1·08 per cent using POSSUM[2] and 0·2 per cent for P-POSSUM[7]. Even the latter is too high, as the risk of death for a fit patient undergoing minor or intermediate surgery approaches zero[15,16]. The lowest possible predicted chance of death with the SRS is 0·07 per cent.

In this study, all patients under the care of the index surgeon who had an operation during the study period were included in the results. None was excluded. This has not been the case for many of the audit systems described previously. Klotz *et al.*[3] included elective cases only, Whiteley *et al.*[2] excluded all day-case and paediatric admissions, and Copeland *et al.*[1] excluded all patients who did not require inpatient care for at least 24 h as well as patients undergoing trauma surgery. The present study has clearly demonstrated the accuracy of the SRS in assessment of risk for a broad group of patients undergoing surgery in a district hospital setting, rather than for selected subsets.

One of the principal advantages of the SRS is that it is independent of the surgeon so that differences in surgical care and outcome can be highlighted. Surgeon-dependent variables such as blood loss are excluded. The score is calculated per procedure, rather than for the total admission (as for POSSUM). Thus, a surgeon who undertakes one correct operation will be treated equally with the surgeon who undertakes multiple operations for the same problem. It should be remembered that POSSUM allocates extra points for multiple procedures, potentially hiding technical surgical deficiencies under the 'high risk' banner[17]. The SRS does not contain specific operative details so that it may apply equally well to all areas of general surgery and to other surgical specialties, such as orthopaedics and gynaecology.

The score is not only easy to calculate but also allows clear visualization of the result as there are only 12 possible scores. This may enhance the ability of the surgeon to form a more objective and accurate preoperative assessment of risk, which could then improve the information given to the patient and relatives. However, the SRS is principally a tool for comparative audit and is not designed to affect the decision to operate, a decision that must always remain clinical.

In conclusion, reliable comparative audit is essential as the surgical standards of institutions and individual surgeons come under increasing scrutiny. To date there has not been a system for comparative surgical audit without major flaws. The SRS is easy to use, formulate and interpret, and has been validated in the authors' patient population. It enables the evaluation of surgical practice independently of surgeon-related variables and is potentially applicable across the entire range of surgical specialties.

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Original article

Surgical technique and outcome of gasless video endoscopic transanal rectal tumour excision

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Background: Transanal endoscopic microsurgery (TEM) is unpopular because of its high cost and most surgeons' unfamiliarity with microscopic surgery. This report describes an experience with a modification of TEM, gasless video endoscopic transanal rectal tumour excision (gasless VTEM), which incorporates a standard laparoscopic video camera and requires no carbon dioxide insufflation system.

Methods: One hundred and one patients with 105 rectal tumours underwent gasless VTEM between 1993 and 2000.

Results: Histological examination revealed 18 adenomas, 75 carcinomas (Tis, 47; T1, 23; T2, five), 11 carcinoid tumours and one lymphoma. The median height above the dentate line and maximum tumour diameter was 5·0 (range 2–14) cm and 2·0 (range 0·4–8·0) cm respectively. The peritoneal cavity was opened intraoperatively in two patients. The median operating time was 53 (range 15–202) min. Bleeding, suture dehiscence and transient incontinence developed after operation in four patients. There was no operative death. Median hospital stay was 5 (range 1–21) days. Eleven patients with T1/T2 staging underwent subsequent radical resection. The median duration of follow-up was 52·3 months. One patient with a carcinoma developed a recurrence.

Conclusion: Gasless VTEM is a feasible, safe and minimally invasive procedure for the treatment of selected rectal adenomas and early carcinomas. The suggested modifications may make the procedure more widely available.

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Introduction

Transanal endoscopic microsurgery (TEM) is an endoluminal, minimally invasive technique for local excision of rectal tumours that was first developed by Buess *et al.*[1–3] in 1984. The major difference between TEM and the conventional trans-sacral or transabdominal approach is that there is no skin incision. Consequently, there is less patient discomfort, faster recovery and lower morbidity. The excellent tumour visualization provided by the endoscopic approach enables precise excision of the tumour[3].

Unfortunately TEM has not gained widespread popularity[4,5]. Its major disadvantages include the high cost of the original instrument set, unfamiliarity of most gastrointestinal surgeons with microscopic surgery, and the awkwardness and complexity of operating the TEM system[4,5]. In addition, the original apparatus, including a combined endosurgical unit (that regulates carbon dioxide insufflation, irrigation and suction) and binocular stereoscopic eyepiece system, is relatively expensive.

More recently, gastrointestinal surgeons have become increasingly well trained in videoscopic surgical techniques. It has been shown that general laparoscopic instrumentation (a standard laparoscope, video camera and standard laparoscopic instruments) can be used for TEM. Swanstrom *et al.*[5] reported a technique of video endoscopic transanal rectal tumour excision (VTEM), which uses a modification of the TEM instrument that incorporates a standard laparoscope and video camera as well as standard laparoscopic instruments.

In this paper development of a modified VTEM (gasless VTEM) is reported, in which a standard laparoscopic video camera is used instead of the binocular stereoscopic eyepiece and carbon dioxide insufflation is not required. The surgical technique of gasless VTEM is described and early experience is reported.

Patients and methods

Patient selection

One hundred and one patients with 105 rectal tumours underwent gasless VTEM between September 1993 and March 2000 at Nagasaki University Hospital and Sasebo Municipal General Hospital. There were 69 men and 32 women, of median age 64 (range 36–92) years. Three other patients, initially selected for gasless VTEM, were found to be unsuitable at the time of operation because of tumour height. These patients had laparotomy and partial resection of the rectum.

The indications for gasless VTEM were: (1) sessile Tis or T1 tumours deemed unsuitable for complete excision by endoscopic snare diathermy; (2) T2 cancers (before endoscopic ultrasonography became available in three patients and in two poor-risk patients); (3) carcinoid tumours; and (4) residual tumour after endoscopic snare diathermy. No patient received preoperative or postoperative chemotherapy or radiotherapy. Patients who did not consent to the procedure and those with synchronous cancers were excluded from the study.

Gasless VTEM was performed by two surgeons who had received no training in the TEM technique. A prospective registry was instituted in which patient and tumour demographics were recorded. A minimum of 1 year of follow-up was available for all patients.

Operative technique

All patients underwent preoperative colonoscopy with biopsy of the tumour and barium enema to confirm tumour location. Most of the cancers were evaluated with endoscopic ultrasonography for preoperative staging. Some 2–3 litres polyethylene glycol electrolyte solution, given 1 day before operation, served as bowel preparation.

Operations were usually performed under spinal anaesthesia. General anaesthesia was selected for patients who had more than one tumour or a large tumour occupying more than one-third of rectal circumference, and when the surgeon estimated an operating time of 2 h or more. The position of the patient during operation was dependent on tumour location. Because the bevel of the rectoscope must face downward, patients were positioned accordingly: the patient was placed in the lithotomy position for a posterior or lateral lesion and in the prone position for an anterior lesion.

The equipment consisted of a rectoscope tube (12 or 20 cm long, 40 mm wide) with a face-piece, a supporting device that was attached to the operating table, and operating instruments (angled tissue graspers, high-frequency cautery knife and injection needle) (Richard Wolf, Knittlingen, Germany)$^{1-3,6}$. The combined endosurgical unit for carbon dioxide insufflation, irrigation and suction, and the binocular stereoscopic eyepiece system were not used. A standard laparoscopic video camera was used in place of the stereoscope (*Fig. 1*).

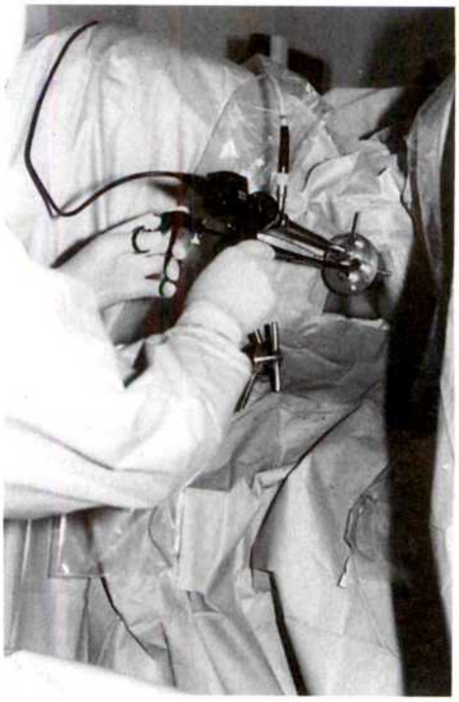
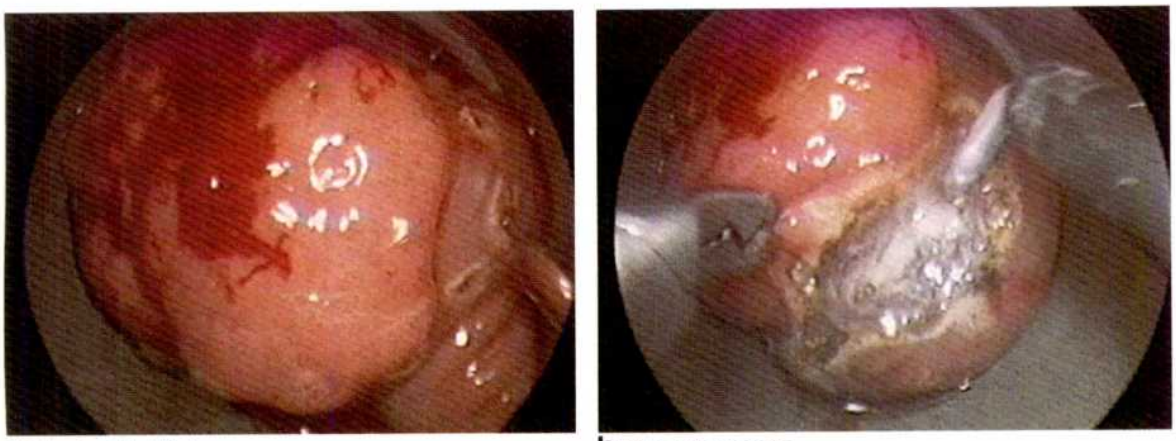


Fig. 1 Operative system for gasless video endoscopic transanal rectal tumour excision

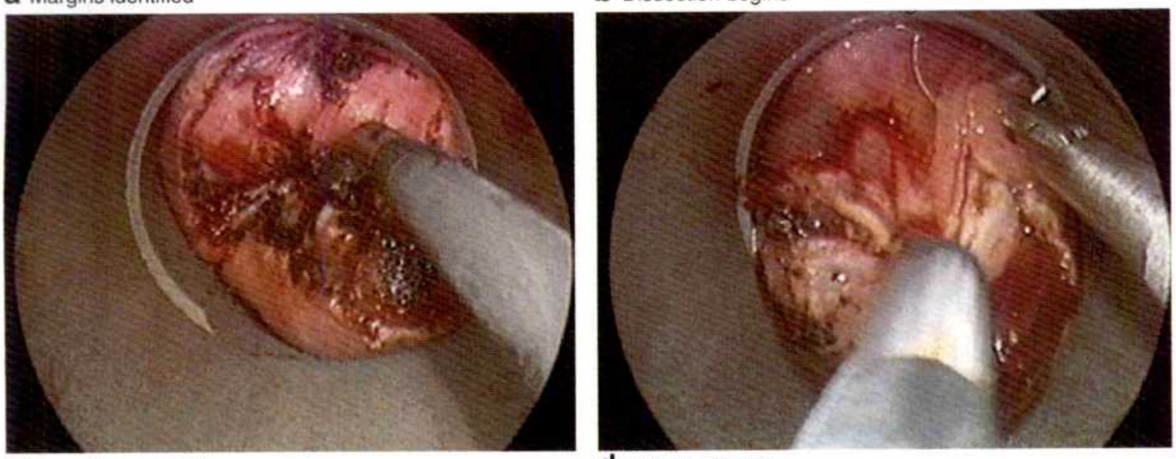
Gentle rectal dilatation was performed and a rectoscope of appropriate length was introduced under direct vision to the desired location so that the lesion occupied the centre of the field of view. The rectoscope was then secured to the operating room table by a supporting arm so that the bevel of the rectoscope softly touched the mucosal surface. A standard 10-mm laparoscope was connected to a video system to allow an adequate view of the operating field (*Fig. 1*). As carbon dioxide insufflation was not used during the operation, the face-piece with four ports did not have to be sealed by capped rubber sleeves. A needle-tip knife was used to outline circumferentially a 5-mm margin of normal mucosa. Adrenaline 1 : 50 000 solution was injected into the submucosal layer for haemostasis (*Fig. 2a*).

Rectal adenomas and Tis cancers were resected by mucosectomy. T1 cancers, carcinoid tumours and residual tumours after endoscopic snare diathermy were resected by mucosectomy or partial wall excision, and T2 cancers were resected by full-thickness excision. Mucosectomy, partial



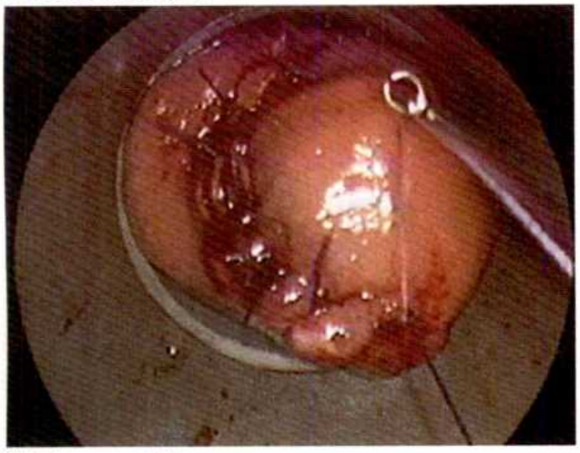
a Margins identified

b Dissection begins



c Resection complete

d Suturing begins



e Suture complete

Fig. 2 Procedures for gasless video endoscopic transanal rectal tumour excision. **a** A needle-tip knife was used to outline circumferentially a 5-mm margin of normal mucosa; adrenaline 1 : 50 000 solution was injected into the submucosal layer for haemostasis. **b** The tumour edge was grasped, elevated and separated from the underlying muscularis. **c** Resection of the tumour completed. **d** and **e** Primary closure with a knotted 3/0 absorbable suture

wall excision and full-thickness excision were defined according to Buess *et al.*[7] During resection the tumour was grasped, elevated and separated from the underlying muscularis (*Fig. 2b* and *c*) or extrarectal fat. In resecting a large lesion, the rectoscope was moved to keep the operative field in the centre of the visual field. After irrigating the wound with saline, primary closure was accomplished with a knotted 3/0 absorbable suture using a needle-holder and a string-knotting device (Mizuho, Tokyo, Japan) (*Fig. 2d* and *e*).

Pathological evaluation

Immediately after gasless VTEM, resected specimens were spread on a cork specimen board by means of fine needles to avoid anatomical distortion by formalin fixation. Pathological examination of the resected specimen was done using stepwise parallel sections measuring 4 mm in thickness, each of which was stained with haematoxylin and eosin and elastica van Gieson. Pathological analysis of rectal cancers included depth of cancer invasion, presence or absence of lymphatic or vascular invasion, and tumour differentiation. Based on histological evidence, resection of each tumour was reported by the pathologist as either complete or incomplete according to the extension of the tumour to the resection margin[8,9]. American Joint Committee on Cancer classification and stage grouping were used to classify the tumours[10]. Each tumour was classified histopathologically according to World Health Organization criteria[11].

A subsequent radical rectal excision (low anterior resection or abdominoperineal resection with lymph node dissection) was recommended for patients whose specimens resected by gasless VTEM revealed the following pathological findings: (1) T1 carcinoma with lymphatic or vascular invasion, poor tumour differentiation or mucinous adenocarcinoma, and/or carcinoma infiltrating more than half the submucosa[12]; or (2) T2 carcinoma.

Table 1 Postoperative histology of tumours treated by gasless video endoscopic transanal rectal tumour excision

	No. of lesions
Adenoma	18
Adenocarcinoma	
Tis	47
T1	23
T2	5
Carcinoid tumour	11
Lymphoma	1
Total	105

Follow-up

The patients were examined at 3-month intervals for the first 3 years after operation and every 6 months thereafter. Complete clinical examination, digital rectal palpation and sigmoid colonoscopy were performed. In addition, patients operated on for cancer underwent periodic evaluation for metastatic disease. Median length of follow-up was 52·3 (range 12·2–91·1) months. No patient was lost to follow-up.

Results

Of the 101 patents, 97 had a single rectal tumour and four had two tumours. Of the 105 rectal tumours, 62 tumours were removed by mucosectomy, 37 tumours by partial wall excision and six by full-thickness excision. Eighteen lesions

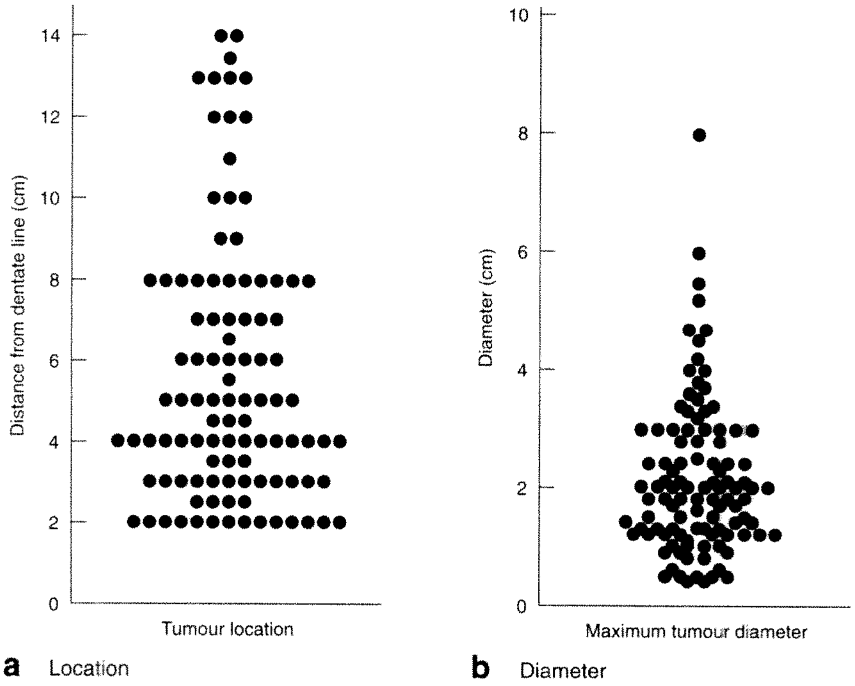


Fig. 3 a Individual tumour distance from the dentate line. b Individual maximum tumour diameter

had a postoperative histological diagnosis of benign adenoma. There were 75 adenocarcinomas (Tis, 47; T1, 23; T2, five), 11 carcinoid tumours and one lymphoma (*Table 1*). The median height of the tumour above the dentate line was 5·0 (range 2–14) cm and the median maximum diameter of the tumour was 2·0 (range 0·4–8·0) cm (*Fig. 3*). The tumour position within the rectum was classified as posterior in 49, anterior in 29, left lateral in 12 and right lateral in 15.

Intraoperative course

Eighty-five patients underwent gasless VTEM under spinal anaesthesia, while 16 patients required general anaesthesia. Intraoperative complications occurred in two patients, in whom the peritoneal cavity was opened. In both patients the tumour (Tis) was located above the peritoneal reflection and the operation was converted, with complete excision of the tumour. Excluding these two patients, the median operating time and blood loss was 53 (range 15–202) min and 5 (range 0–450) ml respectively. The median operating time was 60 (range 25–191) min for mucosectomy, 43 (range 15–202) min for partial wall excision and 75 (range 40–120) min for full-thickness excision.

Postoperative course

There was no operative death. Four patients (4 per cent) developed postoperative complications: bleeding (two), suture dehiscence (one) and transient incontinence to liquid and gas (one). These were managed conservatively. The median length of hospital stay was 5 (range 1–21) days.

Adenomas

Eighteen patients had a postoperative diagnosis of benign adenoma, and in all complete excision of the tumour was confirmed histologically. Microscopic examination showed that the median margin of clearance was 0·6 (range 0·5–2·4) mm in depth and 2·6 (range 1·8–4·1) mm in width. No patient with adenoma developed a tumour recurrence.

Carcinomas

Seventy-five patients had a postoperative diagnosis of well or moderately differentiated adenocarcinoma. In all complete excision of the tumour was confirmed histologically. Microscopic examination showed that the median margin of clearance was 1·0 (range 0·6–5·6) mm in depth and 3·4 (range 2·0–5·5) mm in width. Of the 11 patients with a T1 tumour in whom there was an indication for additional radical operation, eight underwent low anterior resection with lymph node dissection and three refused additional operation. Of five patients with a T2 tumour, two underwent low anterior resection, one abdominoperineal resection with lymph node dissection, and two elderly poor-risk patients were followed without additional surgery.

One patient with a Tis carcinoma (well differentiated adenocarcinoma without lymphatic or vascular invasion) developed a local tumour recurrence that was completely resected by reoperation using gasless VTEM 26 months after the initial surgery.

Discussion

Although the original TEM developed by Buess *et al.*[1–3,7] is patient friendly, it is not user friendly[5]. The major disadvantages of the original procedure are that it is technically demanding and complex to set up. Microscopic surgery using the binocular stereoscopic eyepiece system is unfamiliar to most gastrointestinal surgeons. In addition, the apparatus is relatively expensive. Recently, the videoscopic surgical technique has become more familiar, easier to learn, and is more widely accepted by general and colorectal surgeons and their nursing staff. Swanstrom *et al.*[5] reported a modification of the TEM technique (VTEM) that incorporates a standard laparoscope and video camera as well as standard laparoscopic instruments. Use of an already available laparoscope and laparoscopic instruments decreased the initial cost of the set-up from US $68 000 to US $39 000[5]. Gasless VTEM also uses a standard laparoscope and video camera instead of a binocular stereoscopic eyepiece system. Not only is the procedure feasible and safe, but the ergonomics of the system and the large-screen view obtained make the procedure comfortable for the surgeon[5]. The original TEM also requires carbon dioxide insufflation and use of special instruments (the combined endosurgical unit) that regulate carbon dioxide insufflation, irrigation and suction. On the other hand, the gasless VTEM described in this study is performed without carbon dioxide insufflation. This adaptation further decreased the cost of the set-up from US $39 000 to US $19 200.

Use of gasless TEM has been described previously[13–15]. Kakizoe *et al.*[14] and Yamashita *et al.*[15] reported rectal tumour resection by gasless TEM using a newly developed rectoscope. Ikeda *et al.*[13] also reported on three patients with T1 rectal tumour successfully resected by TEM, using a design similar to that currently described.

The surgical outcomes (operating time, blood loss, operative mortality and morbidity rates, and tumour size) achieved with gasless VTEM were similar to those reported using the original TEM[6,16–19]. However, tumours in the distal sigmoid colon (up to 22 cm from the anal verge) can also be resected by latter technique[16], whereas it is not possible to resect tumours above 14 cm with gasless VTEM. Carbon dioxide insufflation in the original procedure may broaden the surgical field and allow resection of the tumour in the distal sigmoid colon. In the present series, excision by gasless VTEM in three patients proved impractical at the time of surgery, as the rectoscope could not reach tumours located high on the anterior wall. Such patients may have been suitable for TEM with carbon dioxide insufflation.

Buess *et al.*[3,20] identified two subgroups of patients suitable for TEM excision: patients with a good chance of curative treatment with T1 low-risk carcinoma and patients with a limited chance of curative treatment with T2 low-risk carcinoma[21,22]. The authors agree with the indications reported by Buess *et al.*[3,20] if the latter indication is reserved for elderly or high-risk patients. TEM should be restricted to patients with T1 low-risk carcinoma[20]. In the current study, to date, only one patient with Tis carcinoma has developed a tumour recurrence at the resection site. This may have been due to an incomplete margin of excision.

The gasless VTEM presented in this study is a feasible, safe and minimally invasive procedure for the treatment of carefully selected rectal adenomas and early carcinomas. The advantages of gasless VTEM are the low cost of instrumentation, the ease of set-up, and the familiarity of surgeons with video-assisted surgery. These modifications have made TEM more widely available to more surgeons, and potentially the treatment of choice for selected patients with rectal adenoma and early carcinoma.

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Comparative anatomical study of division of the ileocolic pedicle or the superior mesenteric pedicle for mesenteric lengthening

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Background Lengthening of the mesentery by vascular division may be necessary to perform an ileal pouch–anal anastomosis without tension. The aim of this study was to compare, in fresh cadavers, the increase in mesentery length after division of the ileocolic pedicle (ICP) and the superior mesenteric pedicle (SMP).

Methods Total colectomy was performed in 12 fresh cadavers, which were then randomly divided into two groups. Pouch–anal anastomosis was performed with division of the ICP in one group of six cadavers and with division of the SMP in the other. The ileum was measured and the increase in length was recorded and compared statistically.

Results The mean(s.d.) increase in length was 3·0(0·8) cm after ICP division and 6·5(1·1) cm after SMP division ($P < 0{\cdot}001$). The distance between the end of the ileum and the point giving the greatest length was 25·5(5·0) cm in the ICP group and 46·8(4·2) cm in SMP group ($P < 0{\cdot}001$).

Conclusion In fresh cadavers, the increase in mesenteric length was greater after SMP division than after ICP division, but if pouch–anal anastomosis is performed a short segment of small bowel must be removed.

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Introduction

Since its initial description in 1978[1], restorative proctocolectomy with ileal pouch–anal anastomosis has become the procedure of choice for patients with chronic ulcerative colitis or familial adenomatous polyposis. The J pouch is the most commonly used configuration[2], and only the choice between a stapled or handsewn anastomosis remains controversial[3,4].

Pouch–anal anastomosis may be difficult when the small bowel mesentery is too short. In such cases, several techniques for lengthening the mesentery have been proposed, including vascular division. Division of the ileocolic pedicle (ICP) is probably the most commonly used[5,6]. If the ileum did not initially reach the anal canal, the authors have divided the superior mesenteric pedicle (SMP) high in the mesentery, a manoeuvre previously shown to be safe with no effect on functional outcome after pouch–anal anastomosis[7].

The aim of this study was to compare, in fresh cadavers, the increase in length of the mesentery after division of the ICP and after division of the SMP.

Materials and methods

This study was performed in the Anatomical Laboratory of the Paris School of Medicine on 12 fresh cadavers. None of these cadavers had previously undergone abdominal surgery.

Surgical procedure

Total colectomy was performed through a midline and transverse laparotomy. The terminal ileum was divided close to the caecum and the ICP was preserved. The posterior attachment of the entire small intestinal mesentery was mobilized up to the third portion of the duodenum. The inferior part of the head of the pancreas was mobilized.

Methods of measurement (*Fig. 1*)

The point of the ileum that gave the greatest length, the best potential site for ileoanal anastomosis, was marked (I point). The distance between the end of the ileum (E point) and the I point was recorded (EI). The ileum was stretched towards the lower edge of the symphysis pubis (S point) and the distance between the S point and the I point was recorded (SI). If the ileum was above the lower edge of the symphysis pubis, then SI was negative, and if it was below the symphysis pubis SI was positive.

Vascular divisions

The cadavers were randomly assigned to one of two groups. In the first group of six the ICP was divided and in the second the SMP was divided. The ICP was divided at the midpoint between its origin and the marginal vascular arcade (*Fig. 2*). The SMP was divided 2–3 cm after the origin of the ICP (*Fig. 3*). In each group the mesentery was opened extensively beyond the vascular division.

After division of the vessels, the point of the ileum that gave the greatest length had changed; this new point was marked (I′ point). The new distance between the end of the ileum (E point) and the I′ point was recorded (EI′). The ileum was stretched and the distance between the lower edge of the symphysis pubis (S point) and the I′ point was recorded (SI′).

Statistical analysis

The Student's *t* test was used for statistical analysis. $P < 0\cdot05$ was considered to be statistically significant.

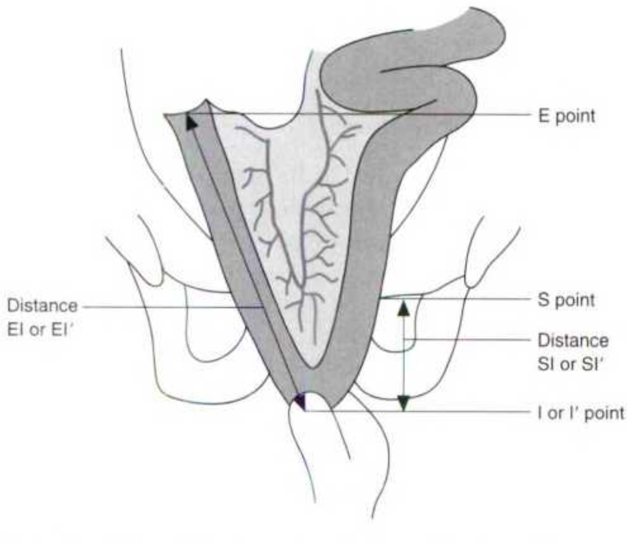

Fig. 1 Stretching of the ileum towards the symphysis pubis

Results

The two groups of cadavers had a similar mean age at the time of death, sex ratio and mean post-mortem delay (*Table 1*).

Before vascular division

The mean(s.d.) distance between the point of the ileum giving the greatest length (I point) and the extremity of the

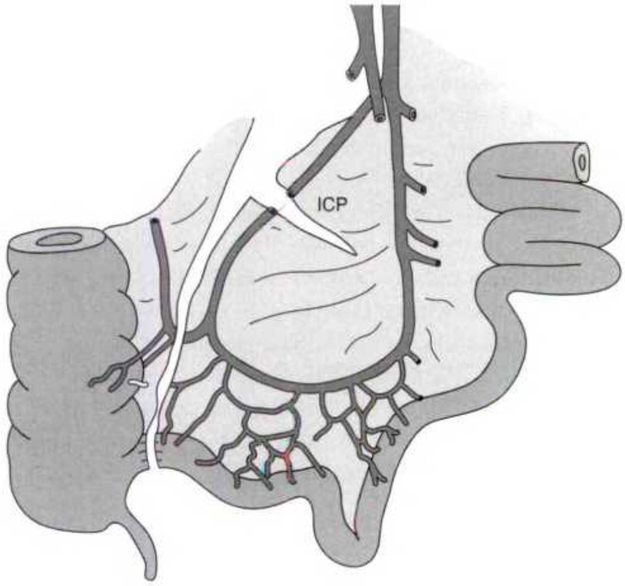

Fig. 2 Division of the ileocolic pedicle (ICP)

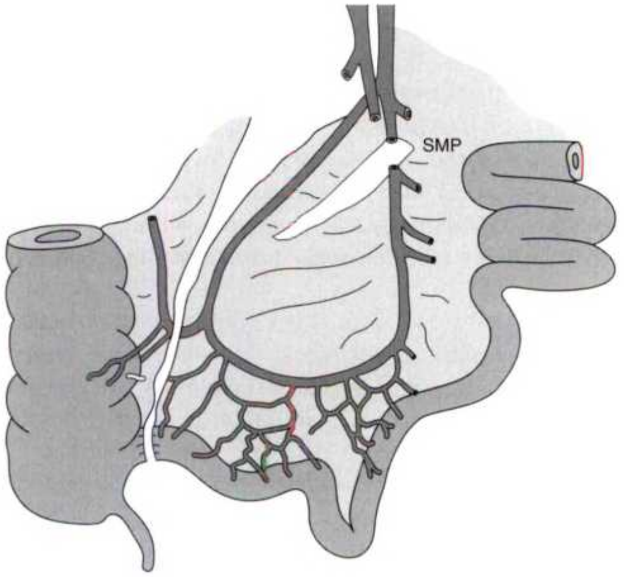

Fig. 3 Division of the superior mesenteric pedicle (SMP)

Table 1 Comparison of cadavers undergoing division of the ileocolic pedicle or superior mesenteric pedicle

	ICP	SMP	P^*
Sex ratio (M : F)	3 : 3	3 : 3	
Age at time of death (years)	84(16)	86(6)	0·8
Post-mortem delay (days)	5(1)	6(3)	0·5

Values are mean(s.d.). ICP, ileocolic pedicle; SMP, superior mesenteric pedicle. *Student's *t* test

Table 2 Changes in measurements after division of the ileocolic pedicle or superior mesenteric pedicle

	ICP	SMP	P^*
EI (cm)	27·3(3·5)	22·0(7·0)	0·13
SI (cm)	0·2(2·7)	1·5(2·8)	0·4
EI′ (cm)	25·5(5)	46·8(4·2)	< 0·001
SI′ (cm)	3·2(2·3)	8·0(2·6)	< 0·01
Increase in length (cm)	3·0(0·8)	6·5(1·1)	< 0·001

Values are mean(s.d.). ICP, ileocolic pedicle; SMP, superior mesenteric pedicle. EI and EI′, distance between the end of the ileum and the point of the ileum giving the maximum length; SI and SI′, distance between the point of the ileum giving the maximum length and the lower edge of the symphysis pubis. *Student's *t* test

ileum (E point) was 27·3(3·5) cm in the ICP group and 22·0(7·0) cm in the SMP group (*Table 2*). The mean distance between the I point and the lower edge of the symphysis pubis (S point) was 0·2(2·7) and 1·5(2·8) cm respectively (*Table 2*).

After vascular division

The new point of the ileum giving the greatest length (I′ point) was very close to the original I point after ICP division (EI′ = 25·5(5) cm). In contrast, the I′ point was further than the I point from the S point after SMP division (EI′ = 46·8(4·2) cm) ($P < 0.001$) (*Table 2*). The distance between the I and I′ points was 3·5(2·7) cm after ICP and 24·8(4·5) cm after SMP division.

After vascular division, the mean distance between the I′ point and the lower edge of the symphysis pubis (S) was 3·2(2·3) cm after ICP division and 8·0(2·6) cm after SMP division ($P < 0.01$) (*Table 2*). The mean increase in mesentery length was 3·0(0·8) cm after ICP and 6·5(1·1) cm after SMP division ($P < 0.001$) (*Table 2*).

Discussion

The blood supply of the terminal ileum arises from the terminal branches of the superior mesenteric vessels. These terminal branches classically form anastomoses with the marginal vascular arcade of the right colon, which itself originates from the ileocolic artery and the right colic pedicle, and forms anastomoses with branches of the inferior mesenteric pedicle. If there is sufficient flux and the marginal vascular arcade is preserved, vascularization of the terminal ileum may theoretically be achieved by only one of the pedicles or by the arcade itself.

During pouch–anal anastomosis, simple manoeuvres to increase the length of the mesentery are performed, such as moving the posterior attachment of the entire small bowel mesentery up to the third portion of the duodenum, exposure of the inferior portion of the head of the pancreas and division of the peritoneum of the mesentery on the anterior and posterior sides[8]. If such manoeuvres are insufficient to achieve anastomosis without tension, vascular division is necessary. This is particularly true if the mesentery is short, as in an obese patient with a narrow pelvis, or if there is a desmoid tumour in the mesentery.

Division of two or three distal branches is thought to be hazardous owing to the risk of segmental necrosis of the terminal ileum[6,9]. Proximal division is therefore favoured. Division of the ICP and the right colic pedicle at their origins, with division of the distal third of the SMP and preservation of the marginal vascular arcade of the right colon have been performed in an anatomical study, resulting in a 36·5 per cent increase in the length of the mesentery[10]. The right colic pedicle is not usually preserved during colectomy; the technique most frequently used to lengthen the mesentery is division of the ICP. The authors prefer division of the SMP high in the mesentery, 2–3 cm beyond the origin of the ICP, because in most patients in whom the terminal ileum is stretched at the anus the axis of tension corresponds to the SMP. This manoeuvre is safe if the colour of the ileum remains unchanged after clamping of the SMP for at least 15 min before division, and has no effect on functional outcome after pouch–anal anastomosis[7].

This study shows that in cadavers the increase in length of the mesentery is greater after division of the SMP high in the mesentery than after division of the ICP. However, two points must be emphasized if division of the SMP is to give a true increase in mesentery length: first, the mesentery must be divided extensively around the vascular division and, second, the point of the ileum giving the greatest length must be systematically recalculated (I′ point).

The I point is generally further away from the end of the ileum after division of the SMP. In this anatomical study, the mean distance between the end of the ileum and the I point was 22 cm, whereas after SMP division the distance between the end of the ileum and the I′ point was 47 cm. If the 18 cm of one limb of the future J pouch is subtracted from the 47 cm, this leaves about 30 cm of ileum that must be removed. This resection is, of course, open to criticism because the overall length of small bowel has a major effect on the quality of function after pouch–anal anastomosis. In real surgical conditions (living patients), the overall length of the small bowel is variable owing to contraction of the muscular intestinal wall. However, it is usually less than 5 m, whereas the length of the small bowel in cadavers is generally between 6 and 7 m. After clinical pouch–anal anastomosis with division of the SMP, the length of small bowel removed never exceeds 20 cm.

The volume of stool may be increased in patients with a terminal ileostomy if 20 cm of terminal ileum is lost. In the authors' clinical experience, there was no difference in stool frequency after pouch–anal anastomosis with division of the SMP and the procedure without pedicle division[7]. This was explained partly by the short segment of small bowel that was removed, but also by the function of the pouch, which probably reduced the consequences of the loss of intestine.

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Original article

Adrenal surgery before and after the introduction of laparoscopic adrenalectomy

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Background: The widespread introduction of laparoscopic adrenalectomy (LA) may have broadened the indications for a surgical approach to adrenal lesions. The aim of this study was to evaluate how the introduction of the laparoscopic technique changed the profile of patients referred for adrenal surgery.
Methods: Some 243 patients, who underwent adrenal surgery between November 1986 and December 1999, were divided in two groups: patients in group 1 underwent surgery before the introduction of LA (November 1986 to May 1993) and those in group 2 were operated on after the introduction of LA (June 1993 to December 1999). The groups were compared with respect to gender, age, preoperative diagnosis and lesion size.
Results: There were 88 patients in group 1 and 155 in group 2. The mean number of adrenal operations per month was 1·1 during the first period and 2·0 during the second. No differences were found in gender and lesion size. The statistical analysis showed a significant difference in indications for surgery ($P = 0.005$). The patients in group 2 were older but, after excluding those with metastases, the difference between the two groups was not significant.
Conclusion: The introduction of LA resulted in an increase in the number of patients referred. The criteria for patient selection did not change, but more patients with adrenal metastases and incidentaloma were operated on laparoscopically.

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Introduction

Since its first description in 1992[1], laparoscopic adrenalectomy (LA) has been used with increasing frequency and several studies have documented its superiority over conventional open adrenalectomy in terms of postoperative recovery, hospital stay and overall cost[2–6]. This operation is now considered the gold standard approach for removal of adrenal lesions[7]. Moreover, recent reports[8–14] have suggested that indications for LA may be broadened to include lesions such as phaeochromocytoma, metastases, and large and/or potentially malignant adrenal tumours for which a laparoscopic approach has previously been considered hazardous[15].

One possible concern about the wide application of this minimally invasive technique is that it might result in a marked change in patient selection for surgery, since both patients and endocrinologists tend to prefer surgical treatment. This is of particular concern in the case of small non-functioning incidentalomas for which surveillance is generally indicated[16–19].

The aim of this study was to evaluate whether the introduction of LA has resulted in a significant change in indications and patient selection for adrenal surgery.

Patients and methods

All patients who underwent adrenal surgery in this department between 1 November 1986 and 31 December 1999 were included in the study. The medical records of these patients were reviewed and data were collected on gender, age, indication for surgery (preoperative diagnosis), preoperative estimated size of the lesion (ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI)), surgical approach and procedure, operative time, postoperative stay and complications, and final histology.

The patients were divided in two groups, according to whether they were operated on before or after the introduction of LA in this department in June 1993. Group 1 included patients operated on between 1 November 1986 and 31 May 1993, and group 2 those operated between 1 June 1993 and 31 December 1999. The study interval for both groups was 79 months.

Data for the two groups concerning gender, age, lesion size and preoperative diagnosis were analysed and compared. Statistical analysis was performed by using the *t* test for age, Wilcoxon's unpaired test for lesion size, and the χ^2 test for gender and preoperative diagnosis. JMP® software (SAS Institute, Cary, North Carolina, USA) was used. $P < 0{\cdot}05$ was considered statistically significant.

Results

Between November 1986 and December 1999, 243 patients were operated on for an adrenal disease. Eighty-eight patients (36·2 per cent) were operated on during the first interval and were included in group 1; the remaining 155 patients (63·8 per cent) were operated on during the second interval and were included in group 2. The mean number of adrenal operations per month during the first 79-month period was 1·1 while that during the second interval was 2·0. The ratio between the mean number of adrenal operations per month during the second and the first period was 1·8.

Group 1 included 31 men (35·2 per cent) and 57 women (64·8 per cent) with a mean(s.d.) age of 46·6(13·2) (range 13–80) years. The mean(s.d.) operative time was 154·2(61·1) (range 85–345) min. The mean(s.d.) maximum diameter of all the lesions, estimated by ultrasonography, CT or MRI, was 39·8(32·6) (range 7–170) mm, while that for incidentalomas, which represented 12·4 per cent of all the adrenal tumours diagnosed in the first interval, was 48·6(21·7) (range 40–100) mm. Adrenalectomy was performed in all but one patient who had an inoperable adrenal cortical carcinoma. Postoperative complications were observed in four patients (4·5 per cent) (one retroperitoneal haematoma, one left pneumothorax, one renal failure and one wound infection). Mean(s.d.) postoperative stay in hospital was 6·7(2·8) (range 3–19) days and postoperative recovery was complete (return to work or normal activities) after 18·5(4·3) (range 10–32) days.

Group 2 included 70 men (45·2 per cent) and 85 women (54·8 per cent) with a mean(s.d.) age of 50·4(13·9) (range 13–79) years. The mean(s.d.) estimated maximum diameter of all the lesions was 35·6(19·1) (range 9–100) mm, while that for incidentalomas, which represented 39·3 per cent of all adrenal tumours diagnosed in the second interval, was 45·6(19·5) (range 20–100) mm. A laparoscopic approach was used in 137 patients (88·4 per cent), while open adrenalectomy was performed in 18 (11·6 per cent). Of the latter, four patients presented with a large phaeochromocytoma (greater than 4 cm) that in the early phase of the learning curve was considered a contraindication to laparoscopy, one patient presented with a phaeochromocytoma and associated paragangliomatosis, four patients presented with an adrenal mass that was suspected to be malignant, five patients were excluded on the basis of a medical contraindication to pneumoperitoneum (pulmonary fibrosis in two, cardiac failure in two, coagulation disorders in one) and four refused LA.

Six laparoscopic approaches (4·4 per cent) required conversion to an open operation because of intraoperative cardiac failure (one), diaphragmatic perforation and pneumothorax (one), difficult dissection a schwannoma (one), bleeding (one) and in patients with Cushing syndrome (two).

Adrenalectomy was performed in all but seven patients (4·5 per cent), in whom an explorative laparotomy with biopsy of the lesion was performed because of advanced malignant disease. Mean(s.d.) operative time was 110·9(52·8) (range 30–420) min. Postoperative complications were observed in six patients (3·9 per cent) (bleeding (four), mediastinal emphysema (one) and cerebral ischaemia (one)). The mean(s.d.) postoperative stay in hospital was 4·3(1·7) (range 2–12) days for group 2 as a whole, and 3·8(1·2) (range 2–8) days for those who had a successful LA. The mean(s.d.) time to complete postoperative recovery (return to work or normal activities) in group 2 as a whole was 10·5(3·5) (range 6–15) days.

Table 1 Patient characteristics and preoperative diagnosis in group 1 (1 November 1986 to 31 May 1993) and group 2 (1 June 1993 to 31 December 1999)

	Group 1	Group 2
Sex ratio (M : F)	31 : 57	70 : 85
Mean age (years)*	46·6(13·2) (13–80)	50·4(13·9) (13–79)§
Lesion size (mm)*‡	39·8(32·6) (7–170)	35·6(19·1) (9–100)
Preoperative diagnosis†¶		
Incidentaloma	19 (21·6)	55 (35·5)
Conn's syndrome	34 (38·6)	48 (31·0)
Phaeochromocytoma	19 (21·6)	18 (11·6)
Cushing's syndrome	8 (9·1)	18 (11·6)
Cushing's disease	4 (4·5)	3 (1·9)
Adrenogenital syndrome	3 (3·4)	1 (0·6)
Suspicion of metastasis	0 (0)	12 (7·7)

*Values are mean(s.d.) (range); †values in parentheses are percentages. ‡Evaluated by preoperative ultrasonography, computed tomography or magnetic resonance imaging. §$P = 0{\cdot}04$ *versus* group 1 (*t* test); the two groups are comparable if patients with metastases in group 2 are excluded. ¶$P = 0{\cdot}005$, group 1 *versus* group 2 (χ^2 test)

The demographic characteristics and preoperative diagnoses in the two groups are reported in *Table 1*. The final histological diagnoses are recorded in *Table 2*. There were no statistically significant differences between the two groups with respect to gender and size of the lesion, but there were significant differences in indications for surgery (preoperative diagnosis) ($P = 0{\cdot}005$). Patients in group 2 were significantly older than those in group 1 but, if those with metastases were excluded, the difference between the two groups was not significant (*Table 1*).

Discussion

During the past decade LA has become the accepted standard for adrenal surgery[7] because of its many advantages over conventional surgery in terms of postoperative outcome and overall costs[2–6]. Moreover, the low morbidity rate and early recovery after this operation have led some authors to propose that LA may be performed on an outpatient basis[20].

Safety and shortened hospital stay have made LA more readily accepted by both patients and endocrinologists. However, this raises concern that the indications for adrenalectomy will widen, and result in overtreatment of some patients. Heretofore surgery has been advocated for functioning or suspicious adrenal masses, but the management of clinically silent adrenal lesions (incidentalomas) continues to be debated. Malignancy cannot be excluded completely by imaging studies and so no unequivocal guidelines exist, although most authors advocate surgery only for adrenal lesions larger than 3–4 cm[16–19].

No study has addressed the impact of LA on selection of patients for surgery, in spite of the large number of papers published about this procedure. The results of the present study show that, at least in one department, the introduction of LA has resulted in only marginal changes to the guidelines for surgical intervention. Nevertheless a substantial increase in the number of patients operated on for adrenal disease was noted, from 88 to 155 in two equivalent time periods (*Table 3*). This increase may be explained by an improvement in imaging techniques, which resulted in an increase in the diagnosis of adrenal masses from 190 in the first interval to 220 in the second[16–19,21].

It is reassuring that changes in modalities used to identify patients who presented with an incidentaloma did not result in changes in the size of lesions removed, thus the indications for surgical treatment of an incidentaloma did not change in this unit. All adrenocortical carcinomas either diagnosed or even suspected before operation were excluded from the laparoscopic approach, in accordance with a recent study[22]. In addition, tumours that exceeded 8 cm in largest diameter were not removed laparoscopically. It is concluded that availability of a laparoscopic approach does not lead to surgeons and endocrinologists overtreating their patients.

Adrenal metastases represent a new indication for LA. Oncologists may be prepared to refer selected patients for

Table 2 Histological diagnosis in group 1 (1 November 1986 to 31 May 1993) and group 2 (1 June 1993 to 31 December 1999)

Histology	Group 1	Group 2
Cortical adenoma	43 (48·9)	95 (61·3)
Secreting	32 (74·4)	64 (67·4)
Non-secreting	11 (25·6)	31 (32·6)
Phaeochromocytoma	21 (23·9)	18 (11·6)
Adrenocortical carcinoma	6 (6·8)	7 (4·5)
Myelolipoma	1 (1·1)	3 (1·9)
Cortical hyperplasia	13 (14·8)	12 (7·7)
Adrenal cyst	3 (3·4)	4 (2·6)
Metastasis	–	12 (7·7)†
Other	1 (1·1)*	4 (2·6)‡

Values in parentheses are percentages. *Schwannoma. †Metastasis from lung carcinoma in seven patients (4·5 per cent), colonic carcinoma in one (0·6 per cent), occult malignant neoplasia in one (0·6 per cent) and renal carcinoma in three (1·9 per cent). ‡Lymph node, cavernous haemangioma, schwannoma, neurofibroma (one patient each)

Table 3 Number of patients referred and operated annually

First interval			Second interval		
Year	Patients referred	Patients operated	Year	Patients referred	Patients operated
1986 (Nov)	16	3	1993 (June)	16	13
1987	26	11	1994	32	22
1988	28	12	1995	35	24
1989	27	13	1996	30	22
1990	25	13	1997	36	24
1991	28	15	1998	36	24
1992	26	13	1999 (Dec)	35	26
1993 (May)	14	8			
Total	190	88	Total	220	155

laparoscopic surgery because it is minimally invasive. Considering the excellent compliance shown by these patients and the encouraging results obtained[11,14], this new indication may represent an important advance produced by the introduction of LA. Indeed, in recent years a more aggressive approach to solitary adrenal metastases from non-adrenal cancer in selected patients has been proposed by several authors[23–25].

In conclusion, the introduction of LA resulted in an increase in the number of patients referred, but only in a slight change in the profile of patients who underwent surgery. More patients with incidentalomas and adrenal metastases were selected for surgery, probably because of an improvement in imaging. The mean size of the lesions removed did not change. Although there was a significant difference between the two groups in terms of age, this was accounted for by patients with metastases in the later group.

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Original article

Early reoperation for acute dysphagia following laparoscopic fundoplication

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Background: A small number of patients develop acute severe dysphagia for which reoperation is necessary within 10 days of laparoscopic fundoplication. The aim of this study was to identify clinical variables that might predict the likelihood of this condition occurring, such that it could be avoided in the future.

Methods: This was a prospective cohort study from three tertiary referral centres, using reoperation for acute dysphagia as the main outcome variable. Gastrointestinal symptom rating scale, and psychological well-being index questionnaires were undertaken before laparoscopic fundoplication, and dysphagia scores were determined before operation and 1 year later. Standard preoperative assessment included gastroscopy, oesophageal manometry and pH studies.

Results: Twelve (1·9 per cent) of the 617 patients suffered acute dysphagia, which was predicted by older age and female sex, and resulted in a longer duration of hospital stay. This condition was not predicted by any other demographic, clinical, investigative or operative variables.

Conclusion: The study did not identify useful criteria by which severe acute dysphagia could be anticipated and thereby avoided following laparoscopic fundoplication.

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Introduction

Laparoscopic fundoplication is established as an effective solution to gastro-oesophageal reflux (GOR) disease, and has lower rates of morbidity and mortality than open antireflux surgery[1,2]. Although extensive experience with laparoscopic fundoplication has now been accrued, and medium- to long-term results are good[3–6], in the short term a small number of patients suffer acute total dysphagia. This requires reoperation within days of surgery[6,7].

The aim of this study was to identify any clinical pattern that could predict the likelihood of acute severe dysphagia, in the hope that risk factors could be used to modify the operative procedure and management accordingly.

Patients and methods

From September 1991 to March 1999, data were collected prospectively on 617 consecutive patients undergoing first-time laparoscopic fundoplication for uncomplicated GOR. Indications for surgery included GOR resistant to medical treatment, a desire to avoid long-term medical therapy, and laryngopulmonary symptoms such as chronic adult-onset cough or asthma. Patients were excluded if they had undergone previous antireflux surgery, or if the primary indication was a large paraoesophageal or mixed hernia.

Gastrointestinal symptom rating scale (GSRS)[8] and psychological well-being index (PWBI)[9] questionnaires were undertaken before operation. GOR (DeMeester) symptom scores[10] were the tool by which patients rated their swallowing symptoms on a scale of 0–3 before surgery and at 1 year after operation. It is generally accepted that dysphagia scores of 0 or 1 represent normality, whereas scores of 2 or 3 represent clinically significant dysphagia. All questionnaires were self-administered and collected by an independent research nurse. The patients had at least one preoperative gastroscopy performed, with the worst level of oesophagitis quantified using Savary–Miller scores[11]. Oesophageal manometry was undertaken using a standard, station pull-through technique after a 6-h fast using a Gaeltec system (Dunvegan, Isle of Skye, UK). Oesophageal body peristalsis was measured during ten 5-ml water swallows 30 s apart. Twenty-four-hour pH studies were performed where necessary to confirm the diagnosis of GOR using a naso-oesophageal pH catheter (Zinetics/Medtronic, Skovlunde, Denmark)[12].

Operation was performed by one of four surgeons, all of whom were beyond the learning curve for laparoscopic fundoplication[13], using a standardized technique as described previously[6]. All procedures were performed with posterior crural repair, following division of the short gastric vessels in the majority of cases. Care was taken to ensure a loose, floppy wrap fixed to the oesophagus over a 45–60-Fr intraoesophageal bougie. A number of patients had a concurrent laparoscopic procedure such as cholecystectomy. Selected patients underwent partial fundoplication procedures, either a posterior 200–270° Toupet wrap[14] or an anterior 180° fundoplication[15] if, in the opinion of the operating surgeon, a full wrap might place the patient at high risk of chronic postoperative dysphagia because of oesophageal dysmotility on preoperative manometric testing[16]. Patients were usually discharged on postoperative day 2 when able to tolerate a texture-modified diet.

Patients who suffered early total dysphagia that required reoperation were defined as those unable to swallow liquids within 10 days of the original operation. The decision to reoperate was made on clinical grounds by the operating surgeon.

Comparative results were analysed with SPSS for Windows version 9.0 statistical software (SPSS, Chicago, Illinois, USA) using the Mann–Whitney *U* test for continuous variables and Fisher's exact test for categorical variables, with a two-tailed α level of 0·05.

Results

Of the 617 patients who had laparoscopic fundoplication, 12 (1·9 per cent) suffered acute dysphagia that required reoperation within 10 days. Patients with acute dysphagia were significantly older by a median of 11 years and significantly more likely to be female (*Table 1*). There was no significant difference in the rate of acute dysphagia among patients operated on by each of the four surgeons in the study.

Table 1 Demographic data in 617 patients who had laparoscopic fundoplication

	Acute dysphagia	Normal recovery	*P*†
Sex ratio (M : F)	3 : 9	340 : 265	0·04
Weight (kg)*	72 (56–106)	78 (45–180)	n.s.
Age (years)*	58 (37–76)	47 (1–85)	0·01

*Values are median (range). †Mann-Whitney *U* test. n.s., Not significant

The median duration of preoperative symptoms was not significantly different between patients with acute dysphagia (81 months) and the remainder (60 months). Typical reflux symptoms were the most common indication for laparoscopic fundoplication in this series (548 patients). Sixty-six patients underwent surgery for laryngopulmonary symptoms and two had operation for peptic oesophageal stricture; data for the remaining patient were missing. The indication for surgery was not related to the occurrence of acute dysphagia.

The development of acute dysphagia was also unrelated to the diagnosis at gastroscopy (oesophagitis, stricture, hiatus hernia or Barrett's oesophagus), GOR (DeMeester) symptom score, or GSRS and PWBI results. Patients with an abnormal pH study were no more likely to suffer acute postoperative dysphagia, nor were patients with abnormal preoperative lower oesophageal sphincter pressures. Six patients with normal preoperative motility developed acute postoperative dysphagia, as did six patients with preoperative dysmotility (considering all grades of abnormal persitalsis together) (*Table 2*). Although a higher percentage of those with aperistalsis suffered acute postoperative dysphagia, the differences were not statistically significant.

Ten patients suffered acute dysphagia following laparoscopic Nissen fundoplication and had laparoscopic reoperation to a partial fundoplication. One patient whose initial procedure was a partial posterior fundoplication had laparoscopic reoperation to an anterior fundoplication, and another had a 180° anterior fundoplication revised laparoscopically to a 90° left anterolateral fundoplication. Neither the completeness of the initial fundoplication, the duration of surgery, nor division of the short gastric vessels had a statistically significant impact on the outcome with respect to acute dysphagia (*Table 3*). Among patients who had concurrent cholecystectomy, none developed acute dysphagia. Twelve patients had a laparoscopic procedure converted to an open fundoplication, but none subsequently developed

Table 2 Preoperative oesophageal motility in 598 patients before laparoscopic fundoplication

	Acute dysphagia	Normal recovery
Normal	6 (1·6)	375
Non-specific motility disorder	4 (3·5)	109
Low-amplitude peristalsis	1 (1·1)	88
Aperistalsis	1 (10·0)	10
Not studied	0	4

Values in parentheses are percentages. There was no significant relationship between early dysphagia and preoperative manometric measurements acute postoperative dysphagia. There were no deaths in this series. Patients who developed acute dysphagia that required reoperation had a significantly longer median hospital stay (12 *versus* 3·5 days; $P < 0·001$). At 12-month review no patient had swallowing difficulty after reoperation.

Discussion

Few studies have addressed early complications after laparoscopic fundoplication. Early postoperative morbidity may have a specific cause such as acute paraoesophageal herniation, an overly snug hiatal repair or an unduly tight wrap, or represent general complications such as postoperative haemorrhage[17]. Many surgeons now recognize the important technical elements of fundoplication[18], and have eliminated manoeuvres known to contribute to failure of the procedure; yet there remains a small risk of severe dysphagia that requires reintervention in the early postoperative period. The incidence of this ranges from 1 to 6 per cent[17,19,20], and was 1·9 per cent in the present series.

Although some authors recommend routine early contrast radiology to facilitate identification of patients with total dysphagia[17], in this series the diagnosis was made clinically. Typically, patients are recognized on the first or second postoperative morning holding a dish containing expectorated saliva, and misleadingly may complain of postoperative retching or vomiting. The true diagnosis of complete dysphagia becomes apparent if the patient is challenged with a small glass of water that they are unable to drink. It is important to identify this complication early, ideally within 10 days. Laparoscopic conversion to a lesser fundoplication is a straightforward and uniformly successful exercise at this stage and open surgery is unnecessary[17,19]. Once this early window of opportunity for reoperation is lost, the difficulty and potential complications of laparoscopic reoperative surgery are increased. Lesser degrees of dysphagia, in which patients can tolerate fluids, are common after laparoscopic fundoplication and can confidently be predicted to improve with time. Reassurance is usually the only therapy needed.

This analysis was conducted to identify predictive factors that could be used prospectively to tailor the operative procedure and management to avoid acute severe dysphagia. Statistically, women and the elderly were more likely to develop this complication. It is difficult to express a clinically significant recommendation from these data that could be applied before operation. It was not possible to identify any other clinically useful preoperative variables or intraoperative technical elements that could screen out patients at high risk of early postoperative dysphagia. The data indicate that the complication occurs irrespective of the type of fundoplication performed; even patients undergoing Toupet or anterior partial fundoplication were not exempt from acute dysphagia that required reoperation[15,21]. It is unlikely that an unrecognized technical flaw during the performance of Nissen fundoplication accounts for the problem, unless the fault is common to all three fundoplications. No technical flaws or failures were identified with the original procedure at reoperation in any of the patients in this study. A loose wrap and free fundal mobility was ensured by complete division of the short gastric vessels, although this manoeuvre alone was not observed significantly to affect the rate of acute dysphagia. A large-bore bougie provided assistance in judging the posterior crural closure.

Any concerns about the technical demands of reoperative fundoplication within 10 days were quickly absolved by the observation that minimal adhesions were present, and ports and retraction could be replaced quickly in their former positions. Once the sutures that approximated the initial fundoplication were divided with scissors, the wrap conveniently stayed in position. Blunt dissection was bloodless and allowed the wrap to sit in its new position without the use of graspers, ready for resuturing. The second procedure was completed more quickly than the first fundoplication; thereafter the time course for recovery to hospital discharge was identical to the standard clinical pathway for laparoscopic fundoplication.

Table 3 Operative data in 617 patients who had laparoscopic fundoplication

	Acute dysphagia	Normal recovery
Operation type*		
Nissen	10 (1·8)	544
Toupet	1 (1·8)	55
Anterior	1 (16·7)	6
Duration of operation (min)†	78 (60–275)	105 (48–325)
Short gastric vessels divided	11	567

*Values in parentheses are percentages; †values are median (range). There were no significant differences between groups

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Short note

Limited incision with plastic bag removal for a large fibroadenoma

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Introduction

Large fibroadenomas seldom regress and many continue to grow. Surgical removal by enucleation is still the standard treatment[1].

Several cosmetic procedures have been designed to camouflage the scar by placing the incision in a circumareolar or submammary crease[2]. However, the bigger the tumour, the larger the incision. Removal of the tumour in pieces with a small incision is not acceptable because of the possibility of tumour contamination and recurrence.

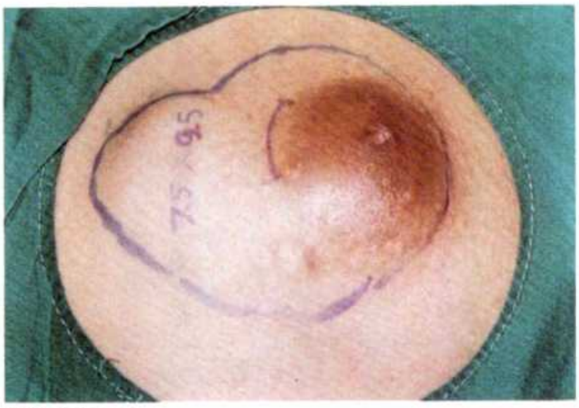
Since October 1997, a plastic bag has been used to collect a large fibroadenoma that has been completely enucleated and removed through a small incision. This technique is simple and creates only a small scar on the breast, so improving the cosmetic result.

Patients and methods

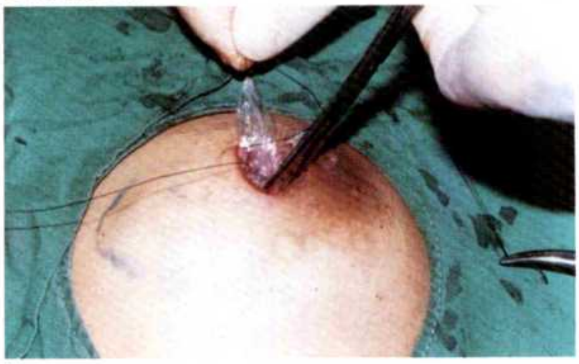
Consecutive patients with a clinical diagnosis of large fibroadenoma greater than 4 cm in diameter had surgery with the new technique. Preoperative ultrasonography and/or core biopsy was done to rule out cystic disease or phyllodes tumour in selected patients. Most had surgery under local anaesthesia; general anaesthesia was used only when patients requested and for very large tumours. Tumour size and weight, length of incision and operating time were recorded. After operation, patients visited the follow-up clinic at 1 week, 1 month and 3–6 months for observation of complications, cosmetic result and any tumour recurrence.

Operative procedure (*Fig. 1a–c*)

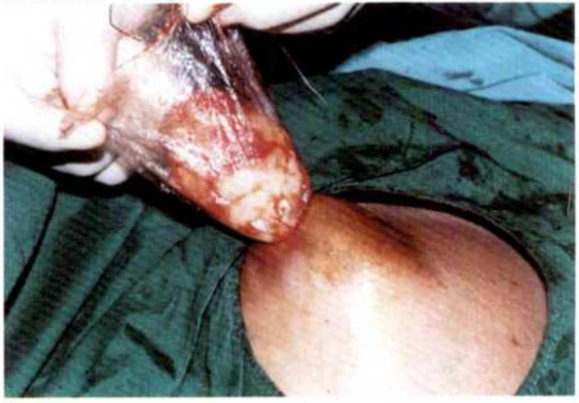
A 2-cm circumareolar incision was made for a central lesion, or a 2-cm curved incision parallel to the areola over the centre of a peripheral tumour. An inframammary incision was used for tumours close to the inframammary crease. After the skin incision, the tumour was enucleated from the breast tissue using a conventional technique. The mass was shelled out completely within the breast. A 10 × 15-cm sterile, transparent plastic bag with three sutures of 2/0 silk tied over the open end in triangular fashion was then inserted through the incision into the cavity. The two



a Incision line



b Insertion of bag



c Tumour removal

Fig. 1 **a** A 2-cm circumareolar incision was made to remove a 7·5 × 9·5-cm fibroadenoma in the right breast of a 19-year-old woman. **b** A 10 × 15-cm plastic bag was inserted through the incision after enucleation was complete. **c** Fragmentation of the tumour was performed within the plastic bag and the tumour was removed through the small incision corners of the open end of the bag were placed beneath the tumour on each side while the other corner was still on the skin surface. By grasping it with an Allis forceps, the tumour was manipulated and put into the plastic bag. Then all the silk sutures at the open end of the bag were raised up over the skin incision. Within the plastic bag, the tumour was divided into fragments with Mayo scissors. The bag was then removed through the incision and the cavity checked for bleeding. No attempt was made to close the space by suturing the breast parenchyma; the skin was closed with subcuticular sutures without drainage. Video-assisted enucleation was helpful for a giant fibroadenoma to avoid over-retraction and pressure necrosis of the wound edge.

Results

Twenty patients with a large fibroadenoma had surgery using this technique. Patient age ranged from 13 to 47 (mean 23) years; 16 patients were less than 25 years of age. Two patients had preoperative ultrasonography and core biopsy for masses of 12 and 5·5 cm in diameter. Mean tumour diameter was 6·3 (range 4–12) cm. Tumour weight varied between 12 and 280 (mean 47) g.

Seventeen patients had operation under local anaesthesia. The other three had general anaesthesia for tumours with a diameter of 6, 6·5 and 12 cm. Two stayed in hospital overnight.

The length of incision was 1·5 cm in two patients, 2 cm in 17, and 3 cm in one patient who had a 12-cm tumour. Operating time (skin to skin) ranged from 25 to 180 (mean 69) min. Video-assisted excision was performed in the patient who had a 12-cm tumour (operating time 180 min).

The operation was accomplished in all 20 patients without complication. Histopathological results indicated the tumour was a fibroadenoma in 17 patients, fibrocystic disease with fibroadenomatous hyperplasia in two and a tubular adenoma in one.

Three patients were lost to follow-up. There were no local recurrences and incisional scars were acceptable after a mean follow-up of 21 (range 6–36) months.

Discussion

Large fibroadenoma and hypertrophic scar are more common in Negro and Oriental races, especially in young adults[2]. For cosmetic purposes, a technique with a small incision should be ideal provided it is simple, safe and has a low tumour recurrence rate.

Sacking the tumour into a plastic bag could succeed only after complete enucleation, which was confirmed by observing the tumour being freely moved within the breast cavity. The transparent plastic bags of 10 × 15 cm used in this procedure are available in most supermarkets and easily sterilized using ethylene oxide.

After removal of the tumour, no attempt was made to obliterate the cavity by sutures and no drainage was used. This was standard practice after a previous prospective randomized trial showed no significant benefit of suture and drainage[3].

Endoscopic breast excision has been reported by some institutions[4]; however, it is difficult and time consuming, and associated with a long learning curve. It also requires general anaesthesia. Video-assisted enucleation may be appropriate with conventional technique and instruments.

A moderately sized phyllodes tumour may be indistinguishable from a large fibroadenoma. Ultrasonography with or without core biopsy should be performed before operation where there is any doubt. However, a preoperative core biopsy for phyllodes tumour may be mistaken as a fibroadenoma. Even if enucleation is performed, expectant follow-up can be continued if phyllodes tumour is benign on histopathological examination[2,5]. A borderline or malignant phyllodes tumour will require further wide local excision or mastectomy[6].

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Original article

Use of the St Gallen classification for patients with node-negative breast cancer may lead to overuse of adjuvant chemotherapy

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Background: The 1998 St Gallen classification was devised to guide clinicians in the use of adjuvant systemic therapy for women with early breast cancer. In this study, the classification was applied to a historical group of patients with node-negative breast cancer who were treated without adjuvant therapy.

Methods: The St Gallen classification was applied to 421 women with breast cancer treated with conservative surgery and radiotherapy alone between 1979 and 1994. Primary tumour characteristics were reviewed centrally.

Results: When the most stringent version of the St Gallen classification was applied (grade 2 or 3 tumours classified as 'high risk'), only 10 per cent of patients were 'low risk', with a 10-year distant relapse-free survival (DRFS) rate of 100 per cent, and 15 per cent were at 'intermediate risk' (10-year DRFS rate of 94 per cent). The high-risk group (75 per cent of women) had a 10-year DRFS rate of 77 per cent ($P < 0.01$). If the St Gallen classification had been applied to all patients in this series who were aged less than 70 years, up to 91 per cent would have been recommended to have chemotherapy.

Conclusion: The St Gallen classification is an inaccurate measure of prognosis for patients with node-negative breast cancer and should be used with caution.

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Introduction

The *Lancet* overview of the benefits of adjuvant systemic therapy reported that cytotoxic chemotherapy and/or hormonal therapy increased disease-free and overall survival for patients with stage I or II breast cancer. Although the proportional reduction in the odds of recurrence or death from adjuvant systemic therapy was similar for patients with node-positive or node-negative disease, the absolute improvement in disease-free and overall survival varied between patient subgroups and with the type of chemotherapy[1,2].

The use of chemotherapy is now justified for selected patients with axillary node-negative breast cancer because of the high risk of development of metastatic disease and the absolute survival benefit of up to 10 per cent[1,2]. However, between 70 and 95 per cent of patients with node-negative breast cancer remain disease-free after local therapy alone at 10 years[3–6]. Therefore, an important objective in the management of node-negative patients is to avoid the morbidity and cost of chemotherapy for patients at low risk of recurrence and to identify high-risk patients who may benefit. This is particularly important for patients with screen-detected breast cancer, many of whom have a good prognosis and in whom the natural history of the disease may differ from that in patients enrolled in the trials included in the *Lancet* overview.

The St Gallen classification and recommendations were developed by a consensus panel of international opinion leaders at the Sixth International Conference on Adjuvant Therapy for Primary Breast Cancer held in St Gallen, Switzerland, in February 1998. Patients were classified into 'low', 'intermediate' and 'high' risk groups based on a combination of axillary lymph node status, tumour size, tumour grade, hormone receptor status and age (*Table 1*)[7,8].

The Editors have satisfied themselves that all authors have contributed significantly to this publication

Table 1 St Gallen classification of patients with primary breast cancer into risk groups

Risk factor	Low risk*	Intermediate risk*	High risk†
Axillary lymph node status	Negative	Negative	Negative or positive
Tumour size (cm)	≤ 1	1–2	> 2
Grade	1	1–2	2–3
Receptor status	ER$^+$ and/or PR$^+$	ER$^+$ and/or PR$^+$	ER$^-$ and PR$^-$
Age (years)	≥ 35	≥ 35	< 35

*All factors must be present; †only one factor required. ER, oestrogen receptor; PR, progesterone receptor

Table 2 Recommendations for adjuvant systemic therapy for node-negative patients aged 70 years or less, outside clinical trials

Menopausal status	Receptor status	Low risk	Intermediate risk	High risk
Premenopausal	ER$^+$ and/or PR^{+*}	Nil or TAM	TAM ± chemo	Chemo† + TAM or chemo or oophorectomy ± TAM
Premenopausal	ER$^-$ and PR$^-$	n.a.	n.a.	Chemo
Postmenopausal	ER$^+$ and/or PR$^+$	Nil or TAM	TAM ± chemo	TAM ± chemo
Postmenopausal	ER$^-$ and PR$^-$	n.a.	n.a.	Chemo
Elderly‡	ER$^+$ and/or PR$^+$	Nil or TAM	TAM ± chemo	TAM ± chemo
Elderly	ER$^-$ and PR$^-$	n.a.	n.a.	Chemo

*Adapted from Zujewski and Liu[8]. ER, oestrogen receptor; PR, progesterone receptor; TAM, tamoxifen; chemo, chemotherapy; n.a., not applicable. †Ovarian ablation and luteinizing hormone releasing hormone analogues were regarded by the St Gallen panel as still being tested in clinical trials. ‡Defined as greater than 70 years of age or as age 65 years or more

However, the basis for this classification was not detailed. Recommendations for adjuvant chemotherapy were based on the three categories of risk, menopausal status and steroid hormone receptor status (*Table 2*).

In this study, the St Gallen classification was applied to a group of patients with node-negative breast cancer treated without chemotherapy to assess the proportion of patients in each risk category, to calculate the 10-year distant relapse-free survival (DRFS) rate and to identify the proportion of patients who would have been recommended adjuvant chemotherapy. All patients in this cohort were treated with conservative surgery and radiation therapy, without chemotherapy or tamoxifen.

Patients and methods

Between November 1979 and December 1994, 1157 patients with Union Internacional Contra la Cancrum (UICC) clinical stage I or II invasive breast cancer were treated at Westmead Hospital. A stage I tumour was 2 cm or less in diameter with no clinical evidence of axillary lymph node involvement (T1 N0). A stage II tumour was 2 cm or less in size with clinically involved axillary lymph nodes, larger than 2–5 cm with, or without, clinically involved axillary nodes (T2 N0, T1–2 N1), or more than 5 cm without clinically involved nodes. Patients who presented with simultaneous bilateral tumours were excluded from the study. Of the 917 patients who underwent an axillary dissection, 611 patients (67 per cent) had no involved nodes on pathological examination. No systemic therapy was given to 545 (89 per cent) of these patients. The characteristics of the primary tumours of 448 node-negative patients who did not receive systemic therapy (82 per cent) were reviewed centrally by two senior breast pathologists. The slides and/or tumour blocks of the remaining 97 patients (18 per cent), who mostly were operated on in other parts of the country or overseas during the early years of the study, could not be retrieved. Patients were excluded when fewer than five (18 patients) or an unknown number (nine) of axillary lymph nodes were examined[9]. Hence, 421 patients were included in this series.

The median age of patients at diagnosis was 48 (range 22–89) years. Median follow-up for surviving patients was 85 (53–208) months. Fourteen patients (3 per cent) who were lost to follow-up without evidence of disease were censored at intervals of between 1 and 178 (median 46) months.

All patients underwent conservative surgery and adjuvant breast radiation therapy. Details of treatment have been described elsewhere[10]. The extent of axillary dissection varied among the surgeons; the median number of axillary lymph nodes removed was 16 (range 5–49) in patients with five or more nodes included. The median number of slides reviewed per case was 14 (range 1–71). Pathological features examined included histological type (based on the World

Health Organization classification); histological grade of differentiation (based on a modified Scarff–Bloom–Richardson classification)[11]; nuclear grade (I, well differentiated; II, moderately differentiated; III, poorly differentiated); lymphovascular invasion (present, absent or indeterminate); necrosis (absent, minimal, moderate or marked); and margins of excision. Pathological tumour size was defined as the diameter of the invasive component and this was obtained retrospectively from the pathology report or received slides. Patient and tumour characteristics for the study group are shown in *Table 3*.

Oestrogen receptor (ER) status was not determined routinely, particularly in the early years of the study; it was known in only 261 patients (62 per cent). The 10-year actuarial DRFS rate was 77 per cent for patients with ER-negative tumours, 85 per cent for those with ER-positive tumours and 81 per cent for those with unknown ER status. The exclusion of 40 per cent of the women in the study because of unknown ER status would introduce selection bias. In view of the similarity in actuarial DRFS between the ER-positive and ER-unknown women, these two categories were combined, producing an actuarial 10-year DRFS rate of 83 per cent. The potential consequence of this combination might be to reduce survival in the low-risk group and to inflate survival in the high-risk group. Misclassification bias from this combination could only underestimate the findings. In the analysis, patients whose tumours had different ER and progesterone receptor (PR) status were not treated separately[12].

The endpoint used in this study was DRFS rate. An event was scored for any relapse outside the treated breast, including isolated axillary recurrences ($n = 3$). All distant relapses were analysed as failures, irrespective of the status of the treated breast. Patients were censored from the calculation of distant failure, at the time of last follow-up, scoring failure at the time of first discovery of metastatic disease. The development of an isolated contralateral breast cancer without evidence of distant relapse concurrently, at last follow-up or death not related to breast cancer, was not classified as an event ($n = 8$).

The 10-year DRFS rate for each of the three St Gallen risk categories was calculated. In the St Gallen classification, axillary node-negative patients aged 35 years or more with a 1–2-cm tumour, grade 1–2, and ER and/or PR positive, are classified as at intermediate risk. However, a grade of 2–3 alone would also classify the patient as high risk (*Table 1*). Therefore, analysis was performed with patients in the high-risk category who had a grade 2 or 3 tumour, and the analysis was repeated with patients in the intermediate-risk category who had a grade 2 tumour, if all the other criteria for inclusion were satisfied. The potential distribution of patients based on the St Gallen classification, and the consequent recommendations where the risk categories were subclassified by menopausal and steroid hormone receptor status, were analysed. To evaluate the potential proportion of patients in this study who would have received chemotherapy based on the St Gallen recommendation, this analysis excluded patients over 70 years of age, which was the higher of the two definitions of 'elderly' in the St Gallen classification (age greater than 70 years, or more than 65 years)[8].

Table 3 Patient characteristics and 10-year distant relapse-free survival of patients with node-negative T1–2 breast cancer, by risk indicators

	No. of patients ($n = 421$)	Actuarial DRFS rate (10 years) (%)
Age (years)		
< 35	35 (8)	49‡
⩾ 35 (referent)	386 (92)	85
Tumour size (mm)		
⩽ 10 (referent)	102 (24)	96
11–15	133 (32)	85*
16–20	103 (25)	79†
⩾ 21	78 (19)	63‡
Unknown	5 (1)	
Histological grade		
1 (referent)	143 (34)	92
2	139 (33)	80
3	135 (32)	72‡
Unknown	4 (1)	
ER status		
ER^- (referent)	89 (21)	77
ER^+	172 (41)	85*
Unknown	160 (38)	81
ER^+ and unknown	332 (79)	83*

Values in parentheses are percentages. DRFS, distant relapse-free survival; ER, oestrogen receptor. *$P < 0{\cdot}05$, †$P < 0{\cdot}01$, ‡$P < 0{\cdot}001$ *versus* referent (Wilcoxon test)

DRFS analysis was conducted using the actuarial and Kaplan–Meier methods, with Wilcoxon and log rank tests, respectively, for statistical significance. All intervals were calculated from the date of operation. Trend in odds ratios for mortality between the risk categories was determined by means of Cox proportional hazard regression because the data could not be modelled as three categories[13,14]. The Wald test was used for statistical significance ($P < 0{\cdot}05$). All statistical analyses were performed using SPSS statistical software (version 9.0; SPSS, Chicago, Illinois, USA).

Results

After a median follow-up of 85 months, 66 (16 per cent) of the 421 patients developed a distant relapse; 53 (80 per cent) occurred within the first 5 years. The 5- and 10-year DRFS rates were 87 and 81 per cent respectively.

Table 4 Ten-year distant relapse-free survival of patients with node-negative T1–2 breast cancer classified according to St Gallen (1998) risk groups with grade 2 and 3, or only grade 3, tumours in the high-risk group

St Gallen classification	No. of patients	Actuarial DRFS rate (10 years) (%)	Mean DRFS (months)	Trend in odds ratios for distant relapse
Grade 2 and 3 tumours in high-risk group				5.4*‡
Low risk	41 (10)	100	120	
Intermediate risk	63 (15)	94	116	
High risk	317 (75)	77*	103*	
Grade 3 tumours only in high-risk group				5·9†‡
Low risk	41 (10)	100	120	
Intermediate risk	159 (38)	95	116	
High risk	221 (52)	71†	98†	

Values in parentheses are percentages. Low risk: tumour 1 cm or less, oestrogen receptor (ER) positive, grade 1, age 35 years or more. Intermediate risk: classified as neither low nor high risk. High risk: tumour greater than 2 cm, ER negative, grade 2 or 3, or grade 3 only (as indicated), age less than 35 years. DRFS, distant relapse-free survival. ‡Average odds ratio per category (Cox proportional hazard regression). $^*P < 0{\cdot}01$, $^\dagger P < 0{\cdot}001$ *versus* low risk

If the most stringent version of the St Gallen classification was applied, only 41 (10 per cent) of the patients would have been considered to be at low risk, with a 10-year DRFS rate of 100 per cent (*Table 4*). The intermediate-risk category comprised 63 patients (15 per cent) with a 10-year DRFS rate of 94 per cent, not significantly different from that in the low-risk group. The high-risk group accounted for 75 per cent of the women and had a 77 per cent 10-year DRFS rate, significantly lower than that in the low-risk group ($P < 0{\cdot}01$).

When a less stringent version of the St Gallen classification was used such that patients aged 35 years or more with a grade 2 tumour, who also had the other characteristics of the intermediate-risk group, were classified as at intermediate risk rather than high risk, the intermediate-risk group now included 38 per cent of the patients, with a 95 per cent 10-year actuarial DRFS rate and no change in the mean DRFS of 116 months (*Table 4*). The high-risk group comprised 52 per cent of the cases, with a decrease in the 10-year actuarial DRFS rate from 77 to 71 per cent, and in the mean DRFS from 103 to 98 months ($P < 0{\cdot}001$ *versus* low-risk group).

Based on the St Gallen recommendations for chemotherapy in women with node-negative breast cancer, when patients with grade 2 tumours are included in the high-risk category chemotherapy would be administered in either 53 per cent (if all patients who were recommended tamoxifen with or without chemotherapy did not receive chemotherapy) or 91 per cent (if all patient groups that were recommended tamoxifen with or without chemotherapy received chemotherapy). When patients with grade 2 tumours were included in the intermediate-risk category, the corresponding proportions of patients to whom chemotherapy would be given were 42 to 91 per cent respectively (*Table 5*).

Based on the St Gallen classification of treatment recommendations, combining patients in both the low- and intermediate-risk groups, the 10-year actuarial DRFS rate was at least 94 per cent without the use of adjuvant chemotherapy or tamoxifen. The inclusion of patients with a grade 2 tumour in the intermediate-risk group reduced the proportion of women in the high-risk group from 75 to 52 per cent, with a small increase in the 10-year actuarial DRFS rate from 94 to 95 per cent for the intermediate-risk group, and a decline in the DRFS rate from 76 to 71 per cent in the high-risk group.

Discussion

Despite the development of indices that integrate conventional prognostic information[15], the ideal factor or combination of factors that would identify high- or low-risk axillary node-negative patients with breast cancer has not yet been defined[16–21]. This study assessed the value of the St Gallen classification as a means of identifying high-risk patients who may benefit from chemotherapy. The results suggest that the St Gallen classification is an inaccurate measure of prognosis for patients with node-negative breast cancer and, hence, should be used with caution in recommending chemotherapy.

The panel at the 1998 St Gallen consensus conference redefined the threshold for recommending chemotherapy: women with a less than 10 per cent risk of relapse at 10 years were considered to be at low risk and therefore were not considered routinely for chemotherapy. This represented a change from the recommendation of the 1995 consensus

Table 5 Distribution of 409 node-negative patients based on the St Gallen classification and associated recommendations for patients aged 70 years or less, outside clinical trials

	Premenopausal women		Postmenopausal women		
	ER$^+$	ER$^-$	ER$^+$	ER$^-$	Total
Low risk					
Treatment	Nil or TAM	n.a.	Nil or TAM	n.a.	
Percentage treated	5	—	5	—	10
Intermediate risk					
Treatment	TAM ± chemo	n.a.	TAM ± chemo	n.a.	
Percentage treated					
Grade 2 excluded*	8	—	7	—	15
Grade 2 included†	19	—	19	—	38
High risk					
Treatment	Chemo	Chemo	TAM ± chemo	Chemo	
Percentage treated					
Grade 2 included*	32	14	23	7	75‡
Grade 2 excluded†	21	14	11	7	52

*If patients with grade 2 tumours were included only in the high-risk group, a maximum of 91 per cent and a minimum of 53 per cent would receive chemotherapy. †If patients with grade 2 tumours were included only in the intermediate-risk group, a maximum of 91 per cent and a minimum of 42 per cent would receive chemotherapy. ‡Total differs from summed column total due to rounding. ER, oestrogen receptor; TAM, tamoxifen; chemo, chemotherapy; n.a., not applicable

conference where patients with a greater than 10 per cent chance of death (rather than relapse) were considered candidates for adjuvant chemotherapy. The consequence of this change was a lowering of the threshold for recommending chemotherapy.

One of the strengths of this study was that the primary tumour characteristics of all patients were reviewed centrally by two senior pathologists. In addition, the series consisted of women with both screen- and non-screen-detected tumours, as is the case in clinical practice outside clinical trials.

Tumour size, which is one of the most important conventional predictors of distant relapse, is a risk factor in the St Gallen classification[3–6,22–29]. The present data suggest that tumour size is a reasonable criterion for identifying low-, intermediate- and high-risk patients, but there remains the potential confounding effect of a higher proportion of grade 3 tumours as tumour size increases[30]. The survival rates of patients in the present series contrast with the data quoted in the St Gallen conference as evidence that patients with small axillary node-negative tumours have a high incidence of relapse. In the National Surgical Adjuvant Breast and Bowel Project (NSABP) B13 trial, patients with ER-positive tumours measuring up to 1 cm had a 5-year disease-free survival rate of only 77 per cent; the corresponding rate in the NSABP-B14 trial was 82 per cent[8, 31–33]. It must be questioned whether the patients in these trials were truly representative of women with breast cancer seen in a population setting. The basis of the St Gallen recommendation, where a tumour size threshold of 1 cm is used to categorize patients, needs further validation. A particular weakness of the classification is that rarer tumour types associated with a better prognosis are not included. In particular, Rosen *et al.*[34] have argued that, although patients with a pathological tumour size of up to 1·0 cm had a 91 per cent 10-year relapse-free survival rate, patients with special tumour types with a diameter of up to 3·0 cm (medullary, mucinous, papillary, tubular or adenocystic carcinoma) had an 89 per cent 10-year relapse-free survival rate.

Histological grade, an important prognostic factor, is based on pathological assessment of tumour features including degree of tubule formation, number of mitotic figures, and nuclear size and shape. It is also included as a risk factor in the St Gallen classification. However, the subjective nature of the assessment lends itself to inter-observer variability. It may be reliable as a prognostic indicator only if performed by an experienced pathologist. In the present study, central pathology review was conducted by two experienced pathologists and, it could be argued, may not reflect routine clinical practice. Allocation of patients with a grade 2 tumour to the intermediate-risk group could reduce the proportion of patients recommended for chemotherapy with no significant change in the survival of patients in the intermediate-risk category (95 per cent). This is also supported by data from other large series[35].

A controversial aspect of the St Gallen classification is the allocation of women aged under 35 years to the high-risk category. Women diagnosed with breast cancer in their thirties seem to have a poorer prognosis[36]. Young women with breast cancer are more likely to have affected axillary lymph nodes, and to have tumours that are large, high grade and hormone receptor negative. The poor outcome of young women with breast cancer could be attributed to these confounding factors, although many studies have retained a negative effect even after adjustment[37–46]. A recent study from the Danish Breast Cancer Cooperative Group involving more than 10 000 patients with breast cancer found that women younger than 35 years at diagnosis had the worst prognosis of all age groups. The age effect was not significant among women who received adjuvant cytotoxic treatment, but was highly significant among low-risk women who received no adjuvant treatment. It was concluded that all young women with breast cancer should be regarded as high risk and offered adjuvant chemotherapy[47].

The role of steroid hormone receptor status as a prognostic indicator for patients with node-negative breast cancer is difficult to establish because of conflicting data[48–55]. Fisher *et al.*[55] suggested that ER status had less impact on predicting outcome than tumour grade. Others showed that in the first 3–5 years after primary therapy, the prognosis of patients with receptor-positive tumours was better than that of patients with receptor-negative tumours. However, this changed after 5 years so that, eventually, patients with receptor-negative tumours fared better[19]. Hence, at present, the receptor status is used predominantly to help identify node-negative patients likely to benefit from adjuvant hormonal therapy.

The evidence shows that some of the risk factors used in the St Gallen classification and recommendations are reasonable predictors of prognosis. However, if the current St Gallen classification and recommendations had been used in the present study cohort, many patients with a DRFS rate of over 90 per cent, in particular those in the intermediate-risk group, would have received chemotherapy.

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Original article

Role of fine-needle aspiration biopsy and frozen-section evaluation in the surgical management of thyroid nodules

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Background: The role of routine frozen section (FS) in the surgical management of thyroid nodules remains uncertain. This study reviewed the role of FS in the presence of an adequate fine-needle aspiration biopsy (FNAB).

Methods: FNAB and FS were evaluated in 206 patients who had surgery for a thyroid nodule. Cytological specimens were classified as benign, malignant or suspicious. The FS diagnoses were benign, malignant or deferred.

Results: A cytological diagnosis was obtained in 93 nodules; the remaining 113 were classified as suspicious, of which 21 were malignant on definitive examination. The overall accuracy of FNAB was 53 per cent. FS evaluation identified 165 lesions as benign; the diagnosis was deferred until definitive histological evaluation in only eight. The overall accuracy, therefore, was 96 per cent. Routine use of FS was cost-effective; lowering the number of reoperations led to an estimated saving of about 40 per cent.

Conclusion: These data suggest that FS remains an important tool in the surgical management of thyroid nodules and can reduce the number of patients requiring reoperation.

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Introduction

Nodular thyroid disease is a common clinical problem, with a prevalence in the general population ranging from 4 to 7 per cent. However, only 5 per cent of thyroid nodules are malignant[1]. Diagnostic procedures to evaluate thyroid nodules include radionuclide scanning and ultrasonography, but fine-needle aspiration biopsy (FNAB) is the most effective test in selecting the patients who require surgery[2]. Routine use of FNAB has decreased the number of patients treated surgically for benign thyroid nodules[3].

A consensus to a specific surgical approach for suspicious thyroid nodules has not yet been reached. In some centres ipsilateral lobectomy is initially performed in patients with a suspect follicular lesion on FNAB; the lesion is then evaluated by paraffin sections, followed by a second operation for completion thyroidectomy if needed. In the authors' unit, the surgical approach involves ipsilateral lobectomy with routine intraoperative frozen section (FS), in order to plan the extent of thyroid resection.

Because of the diagnostic accuracy of FNAB, some authors suggest that intraoperative FS can be avoided, to reduce the duration and cost of operation[4–9]. Others suggest that thyroid surgery can be planned by evaluating known prognostic factors and intraoperative findings[10–13].

The purpose of this study was to review the role of FS in the surgical management of thyroid nodules, in the presence of an adequate FNAB.

Patients and methods

Patients and cytological specimens

Some 206 consecutive patients (173 women and 33 men) who underwent thyroid surgery at the ear, nose and throat unit of San Luigi Hospital, Orbassano, Turin, between January 1995 and December 2000 were evaluated retrospectively. Their ages ranged between 14 and 89 (mean(s.d.) 49(14); median 49) years. All had one or more palpable thyroid nodules with a diameter greater than 1 cm. Some 108 patients (52 per cent) presented clinically with a multinodular goitre (MNG), while 98 (48 per cent) had a solitary palpable thyroid nodule. Initial evaluation included a complete history, physical examination, measurement of serum-free thyroid hormones and thyrotropin, and neck ultrasonography. All patients included in the study were euthyroid; patients with impaired thyroid function were excluded, even those with suppressed levels of thyroid-stimulating hormone only, to exclude autonomous nodules. Thyroid scintiscan and autoantibody measurements were performed in a few patients only, when clinically advisable.

The indications for operation included a cytological diagnosis of malignancy (14 patients; 7 per cent) or of suspected malignancy (113 cases; 55 per cent). In 79 patients (38 per cent) with benign FNAB diagnosis, surgery was performed for progressive growth, compressive symptoms or at the patient's request.

FNAB and intraoperative FS examination were performed in all patients. The pathologist who reported the FS was blinded to the results of the paraffin sections; the diagnosis of the reported FS was never revised during the retrospective analysis. The cytological specimens from FNAB were classified as: (1) benign, including colloid or hyperplastic nodules, benign cysts and thyroiditis; (2) malignant; or (3) suspicious, including follicular and Hürthle cell lesions.

Frozen-section examination

The FS evaluation was made by the same pathologist. Initially, the specimen was assessed macroscopically (including touch preparation cytology) to select the most significant nodules, to identify widely invasive tumours and to plan the dissection of the surgical sample. The next stage was a histological analysis of between four and ten serial sections. The mean(s.d.) number of sections required to obtain a diagnosis of carcinoma was 6·9(2·1) (median 7). In all cases, ten sections were analysed before concluding that a patient had benign disease. The time required for a careful intraoperative analysis of ten sections was 20–30 min.

Histological criteria of malignancy in follicular and Hürthle cell carcinomas were full-thickness capsular invasion, invasion of vessels located within or immediately outside the capsule, or both. Cytological features were considered in cases of papillary carcinoma[14].

The results of FS were classified as (1) benign, (2) malignant or (3) deferred (inconclusive results).

Table 1 Histological diagnosis in 206 thyroid nodules

	No. of patients
Benign	166 (81)
Nodular goitre	103 (50)
Follicular adenoma	48 (23)
Hürthle cell adenoma	11 (5)
Thyroiditis	4 (2)
Malignant	40 (19)
Papillary carcinoma	14 (7)
Follicular variant of papillary carcinoma	5 (2)
Follicular carcinoma	13 (6)
Hürthle cell carcinoma	8 (4)

Values in parentheses are percentages

Statistical evaluation

The accuracy of FNAB and FS was assessed using definitive histological sections as a reference. Sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) were calculated both for FNAB and FS. Statistical evaluation was performed by means of the χ^2 test.

Results

The histological diagnosis was benign in 166 (81 per cent) of lesions and malignant in 40 (19 per cent) (*Table 1*). Papillary carcinoma was the most common malignancy.

Fine-needle aspiration biopsy

A definitive cytological diagnosis (74 benign, 14 malignant) was obtained in 93 patients (45 per cent). The remaining 113 samples (55 per cent) were suspicious of malignancy. Following histological assessment, 21 (19 per cent) of these were confirmed as malignant lesions (13 follicular and eight Hürthle cell carcinomas). Of the 79 samples initially thought benign, 74 (94 per cent) were confirmed as benign on final pathological examination. The remaining five false-negative lesions (6 per cent) were defined histologically as follicular variants of papillary carcinoma. All 14 patients with a malignant FNAB were confirmed to have a papillary carcinoma (*Table 2*). For statistical evaluation of FNAB, the classification of suspicious was included in the malignant group, because of the effect on the surgical decision; therefore, the number of false-positive results was high (92). The sensitivity of FNAB was 88 per cent and specificity 45 per cent with an overall accuracy of 53 per cent, PPV 28 per cent and NPV 94 per cent.

Frozen-section examination

Frozen-section evaluation suggested that 165 lesions were benign; this was confirmed histologically in 161 (98 per cent), while four (2 per cent) were subsequently recognized as Hürthle cell carcinomas and the patients underwent a second operation. In eight patients (4 per cent) the intraoperative diagnosis was deferred to definitive evaluation. In four these proved to be benign lesions (follicular

Table 2 Comparison of histological results in 206 thyroid nodules with preoperative cytology and intraoperative frozen section

	Final paraffin		
	Benign	Malignant	Total
FNAB			
Benign	74	5	79
Suspicious	92	21	113
Malignant	0	14	14
Total	166	40	206
Frozen section			
Benign	161	4	165
Deferred	4	4	8
Malignant	1	32	33
Total	166	40	206

FNAB, fine-needle aspiration biopsy

adenomas) and four were identified as malignant (three follicular carcinomas and one Hürthle cell carcinoma) (*Table 2*). The latter four patients underwent a second operation.

For statistical evaluation of FS, the uncertain samples were classified as false negative (four carcinomas) or true negative (four adenomas). The sensitivity of FS was 80 per cent and the specificity 99 per cent. A single false positive led to inappropriate total thyroidectomy (the intraoperative diagnosis was follicular carcinoma, but the definitive histological diagnosis was Hürthle cell adenoma). The overall accuracy was 96 per cent, PPV 97 per cent and NPV 95 per cent.

Frozen section in follicular lesions

The accuracy of FS examination was evaluated selectively in 113 patients with a cytological diagnosis of suspected malignancy (follicular lesions). In this series, there were six false negatives (three follicular and three Hürthle cell carcinomas), including four deferred diagnoses. The sensitivity was 71 per cent and the specificity 99 per cent. The overall accuracy of FS for follicular lesions was 94 per cent, PPV 94 per cent and NPV 94 per cent.

Frozen section for solitary nodules or multinodular goitre

The results of FS were compared in patients with a clinically palpable solitary nodule (98 patients; 48 per cent) and those with MNG (108; 52 per cent). There were no significant differences in the accuracy of FS (96 *versus* 95 per cent), sensitivity (81 *versus* 79 per cent) or specificity (100 *versus* 99 per cent). There were four inappropriate operations, followed by a second operation in each group. One patient with a MNG had an inappropriate total thyroidectomy at first operation.

Cost analysis

The cost of both surgery and FS was analysed by means of regional refunding fees to the hospitals. The unitary cost of thyroidectomy and FS was estimated at €2065 and €81 respectively.

The value of routine FS was analysed by comparing the number of second operations needed (eight) with the 26 reoperations required if FS was not done. The total cost of FS in 206 operations was €16 686. The routine use of FS led to eight second operations (four initially thought benign and four deferred on the basis of FS), at a total cost of €16 520. Surgical decisions based on FNAB only would have led to 26 reoperations, at a total cost of €53 690. It was estimated that routine use of FS in the present series led to a saving of €20 484 (excluding the patient who had an unnecessary total thyroidectomy).

Discussion

The use of FS is an important adjunct to the operative management of many forms of cancer. However, in the era of FNAB, the routine use of FS in thyroid surgery may be unnecessary. The economic impact of the introduction of routine preoperative FNAB in the management of thyroid malignancy was illustrated by investigators from the Mayo Clinic, who reported that FNAB reduced the number of patients requiring surgery from 67 to 43 per cent and increased the proportion of surgically proven cancers from 14 to 29 per cent[15]. In the past decade, several reports have debated the routine use of FS[7,10,12,16–21]. Some authors point out that FNAB is complementary to FS in planning the extent of surgery[20]. Others[6,21] suggest that FS can be restricted to cases with an FNAB diagnosis of 'follicular nodule' or 'indeterminate'.

In general, FNAB is considered to be more sensitive and FS more specific for the diagnosis of malignant neoplasm. FNAB is used primarily to select patients for surgery, where a high sensitivity is desirable. FS is employed to plan the extent of surgery, a role requiring a high specificity. In the present study, FS had a good sensitivity (80 per cent) and a high specificity (99 per cent), in agreement with other reports[5,10,16,20,21]. In only eight patients (4 per cent) was the intraoperative diagnosis postponed pending final histological evaluation.

The sensitivity and specificity evaluation of FNAB and FS strictly related to the handling of the respective diagnoses. Because all patients in this study had been selected for surgery, follicular lesions could be false positive or true positive. Furthermore, when FS was inconclusive and led to a conservative operation, these samples could be true negatives or false negatives. These data suggest that FNAB is good for selecting patients who need surgery, but the low specificity and accuracy do not permit adequate planning of the extent of surgery. The low specificity of FNAB could be explained by the large number of MNGs in an area of moderate iodine deficiency; the presence of multiple nodules and a higher percentage of follicular neoplasms may diminish the accuracy of FNAB. On the other hand, FS has a high specificity and led to a reduction in the number of late reoperations from 26 (13 per cent) to eight (4 per cent).

Had the surgical decision been based on FNAB only, 26 of 40 patients with malignancy would have needed a second operation. One Hürthle cell adenoma, diagnosed as a follicular carcinoma on FS, was the only false positive.

The specificity of FS was high (99 per cent) if only the follicular lesions found by FNAB were considered; accordingly, there were few inappropriate operations (seven (6 per cent) of 113). Similarly there was no difference in the accuracy of FS for patients with either solitary nodules or MNG. None of this supports the hypothesis that FS could be used selectively in specific subgroups of patients.

Total or near-total thyroidectomy is the usual surgical treatment for a well differentiated thyroid cancer[22,23]. Routine FS in patients with cancer reduces the discomfort and avoids the costs of reoperation.

In the present series, decreasing the number of reoperations as a result of routine FS led to an estimated saving of about 40 per cent. FS is probably unnecessary when FNAB is diagnostic of malignancy. In this study, the estimated cost saving of avoiding FS in 14 patients with a cytological diagnosis of papillary carcinoma was €1134.

In conclusion, FS evaluation remains a useful and cost-effective tool, complementary to FNAB. FS can be avoided when the FNAB result is consistent with malignancy, but could be of value when the FNAB result is reported as suspicious, inadequate or even benign. The results of both thyroid FNAB and FS are dependent on the pathologist. Each institution should evaluate its own ability to use these modalities in the preoperative and intraoperative assessment of thyroid nodules.

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Original article

Impact of timing on completion thyroidectomy for thyroid cancer

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Background: It has been stated that completion thyroidectomy for thyroid malignancy should be performed either within 10 days of the primary operation or after 3 months, to reduce the incidence of complications. The aim of this study was to review the impact of timing on the rate of complications following completion thyroidectomy.

Methods: Data were obtained retrospectively from the Endocrine Surgery Thyroid Data Base, to which the records of all patients have been entered since 1957. The patients who had the last 100 consecutive completion thyroidectomies for thyroid cancer comprised the study group.

Results: Sixty-three patients had the second operation performed within 10 days or more than 90 days after the initial operation (group 1). Thirty-seven patients had reoperation between 10 and 90 days after the first procedure (group 2). One patient (2 per cent) in group 1 and one patient (3 per cent) in group 2 suffered a permanent complication (*P* not significant).

Conclusion: There was no definite impact of the timing of surgery on the rate of complications after completion thyroidectomy.

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Introduction

Completion thyroidectomy plays an important role in the management of patients with thyroid cancer. Although one-stage total thyroidectomy is preferable, the characteristics of certain histological types of thyroid carcinoma, particularly follicular lesions, render the preoperative and intraoperative diagnosis of malignancy a challenge, even to experienced pathologists[1]. This situation often necessitates a second operation[2].

Completion thyroidectomy carries an inherent risk of complications[3], which is reported to be higher than that associated with the primary operation[4,5]. It is thought that the timing of reoperation is contributory, and that completion thyroidectomy should be undertaken either within 10 days of the initial lobectomy or after 2–3 months, to minimize this risk[1,5–9]. According to Davies[9], completion thyroidectomy is extremely difficult and hazardous to vital structures when attempted after 3–4 weeks, because densely adherent strap muscles make dissection of the contralateral lobe impossible. To date, however, there are no published data to support this argument. There is documented evidence that careful attention to technique decreases morbidity[10–12].

In the authors' unit, completion thyroidectomy is performed at the earliest available time after the definitive diagnosis has been established, with no restriction on timing. The aim of this study was to review the impact of the timing of completion thyroidectomy on the complication rates following operation.

Patients and methods

Data were obtained retrospectively from the Endocrine Surgery Thyroid Data Base. All information relating to operative procedures, pathology and complications has been recorded in this database since 1957. The last consecutive 100 patients who underwent completion thyroidectomy for thyroid cancer comprised the study cohort, spanning the interval from February 1988 to October 2000. Initial operations included ipsilateral total lobectomy, subtotal thyroidectomy and thyroid biopsy. Patients with cancer diagnosed at other centres and referred to this unit for further management were also included. The time interval between the initial operation and completion thyroidectomy formed the basis for comparison of the complications after surgery.

Preoperative vocal cord assessment was done for all patients. Serum calcium levels were determined before operation, on the evening of the operation, and on the first and second postoperative days. Transient hypocalcaemia was defined as that which recovered spontaneously within 6 weeks of operation; temporary recurrent nerve palsy or vocal cord dysfunction should have recovered within 6 months.

Completion thyroidectomy

Completion thyroidectomy was performed through the previous incision; the scar was excised and platysma flaps raised. Dissection was commenced laterally by mobilizing the sternomastoid muscles down to the carotid sheath. Care was taken as the operation proceeded to identify the thyroid lobe or remnant. The strap muscles were then gently eased away from the gland and divided transversely. After this, the remnant tissue was mobilized using the technique of capsular dissection. If a previous total ipsilateral lobectomy had been performed, only contralateral dissection was done. If, however, there was remnant tissue on the ipsilateral side, the remaining gland was carefully excised. The respective external and recurrent laryngeal nerves, as well as the parathyroid glands, were identified and preserved. If there was any doubt regarding the viability of the parathyroid glands, they were transplanted into the ipsilateral sternomastoid muscle. Statistical analysis (odds ratio with 95 per cent confidence intervals) was performed using the SPSS/PC+ (version 9) software package (SPSS, Chicago, Illinois, USA).

Results

The 100 patients were classified according to the time interval between initial operation and completion thyroidectomy. Ninety-one patients had a unilateral total lobectomy, and nine had various subtotal procedures as the primary operation. Sixty-three patients had the second operation either within 10 days of the first operation or after 90 days (group 1). Thirty-seven patients had completion thyroidectomy performed between 10 and 90 days of the initial operation (group 2). The two groups were matched for sex and age (*Table 1*).

Group 1 included 63 patients: 53 who had the repeat operation performed within 10 (median 5) days of the initial operation and ten who had the reoperation more than 90 days later. Six patients had the reoperation several years after the initial operation because of late referral for a second opinion after malignancy was diagnosed, recurrence after subtotal thyroidectomy, review of the need for further surgery, and initial misdiagnosis of a benign adenoma. The other four patients in this group had completion thyroidectomy at intervals of 101, 128, 169 and 183 days. The 37 patients in group 2 had repeat surgery after a median of 15 days.

Table 1 Age and sex distribution of the 100 patients who had completion thyroidectomy

	Group 1 (< 10 or > 90 days)	Group 2 (10–90 days)
Mean (range) age (years)	41 (12–81)	45 (24–89)
Sex		
Women (*n* = 78)	47	21
Men (*n* = 22)	16	6

Complications

There were three complications in 63 patients from group 1: two patients had temporary hypocalcaemia and one had recurrent nerve paresis (*Table 2*). Seven of 37 patients in group 2 had complications: four had temporary hypocalcaemia, two had temporary vocal cord dysfunction and one had recurrent nerve paresis. There was no significant difference between the two groups (odds ratio 0·21 (95 per cent confidence interval 0·04 to 1·04); $P = 0{\cdot}06$). Only two complications were permanent: a recurrent laryngeal nerve palsy in each group (P not significant). In both these procedures the recurrent nerve was sacrificed deliberately to achieve tumour clearance. There were no other long-term complications, no repeat operations and no perioperative deaths.

As a comparison, 205 total thyroidectomies were performed for malignant conditions of the thyroid during the study interval (*Table 2*). Eight of 205 patients who had a total thyroidectomy suffered permanent complications, compared with two of 100 patients who had a two-stage procedure (P not significant).

Discussion

Ideally, surgery for thyroid cancer should be performed as a single procedure. This is not always feasible as malignancy may be confirmed only on examination of paraffin histological sections several days after the primary operation.

There is ongoing controversy regarding the optimal extent of surgery for thyroid cancer. None the less, total thyroidectomy has several benefits[13]: it facilitates detection and ablation of metastatic disease with radioactive iodine,

Table 2 Comparison of complication rates after completion thyroidectomy (n = 100) and total thyroidectomy (n = 205) performed in the same interval

		Completion thyroidectomy	
	Total thyroidectomy	Group 1 (< 10 or > 90 days)	Group 2 (10–90 days)
Nil	172 (84)	60 (95)	30 (81)
Temporary hypocalcaemia	20 (10)	2 (3)	4 (11)
Vocal cord dysfunction	2 (1)	0 (0)	2 (5)
Recurrent laryngeal nerve injury	2 (1)	1 (2)	1 (3)
Haematoma	1 (0)	0 (0)	0 (0)
Reoperation for bleeding	3 (1)	0 (0)	0 (0)
Tracheostomy	2 (1)	0 (0)	0 (0)
Other	2 (1)	0 (0)	0 (0)
Death	1 (0)	0 (0)	0 (0)
Total	205 (100)	63 (100)	37 (100)

Values in parentheses are percentages

allows for monitoring of thyroglobulin levels, eliminates the risk of anaplastic transformation and treats residual cancer in the contralateral lobe, which has been reported to occur in up to 88 per cent of patients[13].

Davies[9] has written that completion thyroidectomy between 10 and 90 days is 'extremely difficult and hazardous to the surrounding structures'. Restriction of the timing of the second operation places significant pressures on the patient and on the healthcare team. Difficulties with diagnosis and the need for several opinions often result in a delay beyond the 10-day deadline. A 3-month wait could impose undue stress on the patient.

This study has shown no significant difference in the incidence of complications from completion thyroidectomy, regardless of the time interval after the initial operation. Several factors contributed to this result. First, a complete ipsilateral lobectomy with isthmusectomy was usually performed at the first operation[9]. If the contralateral lobe was not mobilized, dissection at completion thyroidectomy should be no different from that at first-time hemithyroidectomy as there is minimal tissue reaction. Second, the technique of capsular dissection[10] with careful identification of the recurrent nerves and parathyroid glands reduced the risk of complications in secondary thyroidectomy[11].

The present study has demonstrated that, provided sound surgical principles are adhered to, the timing of completion thyroidectomy may not have any impact on the incidence of permanent complications.

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Short note

Danish nationwide cohort study of postoperative death in patients with liver cirrhosis undergoing hernia repair

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Introduction

Patients with liver cirrhosis and ascites have distension of the peritoneum and subsequent herniation of the weakest structures in the abdominal wall[1].

The risk of 30-day mortality in cirrhotic patients undergoing repair of an umbilical or inguinal hernia was compared to that of a control group.

Patients and methods

This was a follow-up study in Denmark (5·2 million inhabitants) of patients treated from 1993 to 1997. The study cohort was identified from the Danish National Hospital Register, which contains information on all hospital admissions since 1977[2]. Patients were included in the study cohort from the date of the first recorded International Classification of Diseases version 8 diagnosis of alcoholic cirrhosis (571.09), primary biliary cirrhosis (571.90), non-specified cirrhosis (571.92), chronic hepatitis (571.93) or 'other types of cirrhosis, alcoholism not indicated' (571.99)[3].

Patients were identified who had a first-time inguinal (406.20 and 406.40) or umbilical (405.80 and 406.00) hernia repair following the diagnosis of liver cirrhosis. The control group consisted of a random sample of Danish patients over 20 years of age who underwent the same operative procedure on the same day as the patient with liver cirrhosis. A total of 741 patients had inguinal and 102 had umbilical hernia repair. Enrolment was restricted to patients who had a single surgical procedure at the index admission.

Statistical analysis was performed by logistic regression analysis to examine the association between liver cirrhosis and 30-day mortality adjusted for age and type of admission.

Results

A total of 22 840 patients were recorded with a diagnosis of liver cirrhosis. Some 201 of these underwent an umbilical hernia repair and 11 died (5·5 (95 per cent confidence interval (c.i.) 2·3 to 8·6) per cent) within 30 days, compared with one (1·0 (95 per cent c.i. 0·0 to 2·9) per cent) in the control group of 102 patients. The adjusted odds ratio for 30-day mortality was 3·9 (95 per cent c.i. 0·7 to 31·2) (*Table 1*).

Among 256 patients with liver cirrhosis who had an inguinal repair, seven (2·7 (95 per cent c.i. 0·7 to 4·7) per cent) died within 30 days, compared with five (0·7 (95 per cent c.i. 0·1 to 1·3) per cent) of 741 in the control group. The adjusted odds ratio for 30-day mortality was 4·4 (95 per cent c.i. 1·4 to 14·4) *(Table 2)*.

Discussion

Patients with liver cirrhosis undergoing hernia repair had an approximately fourfold increase in postoperative mortality compared with non-cirrhotic patients.

Table 1 Crude and adjusted odds ratios for 30-day postoperative mortality in patients undergoing umbilical hernia repair

	No. of patients	No. of deaths	Odds ratio	
			Crude	Adjusted
Controls (referent)	102	1	1·0	1·0
Cirrhosis cohort	201	11	5·9	3·9 (0·7, 31·2)
Age (years)				
< 65 (referent)	223	10	1·0	1·0
≥ 65	80	2	6·0	5·0 (0·1, 2·6)
Type of admission				
Elective (referent)	193	3	1·0	1·0
Emergency	110	9	5·6	4·6 (1·2, 17·9)

Values in parentheses are 95 per cent confidence intervals

Table 2 Crude and adjusted odds ratios for 30-day postoperative mortality in patients undergoing inguinal hernia repair

	No. of patients	No. of deaths	Odds ratio	
			Crude	Adjusted
Controls (referent)	741	5	1·0	1·0
Cirrhosis cohort	256	7	4·1	4·4 (1·4, 14·4)
Age (years)				
< 65 (referent)	620	3	1·0	1·0
≥ 65	377	9	5·0	4·9 (1·3, 18·9)
Type of admission				
Elective (referent)	884	7	1·0	1·0
Emergency	113	5	5·8	3·7 (1·1, 12·2)

Values in parentheses are 95 per cent confidence intervals

The strengths of this study were the large cohort, the uniformly organized healthcare system in Denmark allowing a population-based design with complete follow-up, and valid data about death. However, there were no clinical details about the indications for operation, anaesthetic procedures used, stage of liver cirrhosis, ascites, smoking habits, alcohol consumption or cause of death.

Case series have shown increased postoperative mortality in patients with liver cirrhosis undergoing umbilical hernia repair, although conclusions vary in studies concerning inguinal hernia[4,5]. Although results for hernia repair were worse in patients with liver cirrhosis, the present data should be interpreted with caution. The indications might have been different in patients with liver cirrhosis compared with controls.

These results are consistent with previous studies indicating an increased postoperative mortality even after simple extraperitoneal surgery in patients with liver cirrhosis.

Acknowledgements

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Prospective analysis of quality of life and survival following mesorectal excision for rectal cancer (*Br J Surg* 2001; 88: 1617–22)

Sir

The quality of life methodology in this study is weak and falls below accepted standards. This renders the conclusions open to criticism. Scientific rigour regarding methodology, results and interpretation is as important in this area as it is in laboratory experiments and clinical trials. Guidelines describing appropriate standards exist[1,2]. We feel that the above study is deficient in a number of areas:

- The justification for the selection of quality of life questionnaires should be given and psychometric properties summarized (the QLQ-CR38 has not been validated in English).
- It is necessary to report both where (in hospital or at home) and who completes the questionnaire. This may influence responses.
- The accepted time window around each assessment should be reported (e.g. 3 months ± 2 weeks).
- Reporting compliance rates, including missing individual questions (item non-response) and missing assessments (unit non-response), is required. Standard methods for inputting missing data exist.
- Reasons for ineligibility or exclusion from quality of life assessments (e.g. death, illiterate) and numbers of quality of life assessments at each time point should be reported.

While the surgical community is largely familiar with the influences of inadequate power calculation or retrospective data analysis, there are issues that are equally important in quality of life studies. Flaws in methodology are not tolerated in other areas of surgical research and the same standard needs to be applied here.

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Present and future management of deep venous thrombosis (*Br J Surg* 2001; 88: 1427–8)

Letter 1

Sir

We have previously demonstrated the safety of withholding anticoagulation in patients suspected of having deep venous thrombosis (DVT) who had a single, negative, complete duplex scan[1]. It was disappointing, therefore, to find that Mr Braithwaite repeats the recommendation that patients who have a negative duplex scan should have that scan repeated after 7 days.

The recommendation that repeat scanning is necessary comes from literature published prior to the use of duplex scanning. The term 'ultrasound scanning' can be applied to a number of different techniques and means different things to different authors. Compression ultrasound uses a B-mode image to visualize the vein and determine whether it is compressible (normal) or non-compressible (abnormal); some studies using this technique applied it at two or three levels only in the leg, excluding the calf. The calf veins were not visualized in these studies, hence the conclusion that rescanning is necessary to ensure that propagation from calf vein thrombosis is not missed[2–4]. Duplex scanning enables the calf veins to be imaged even if compression ultrasonography is inconclusive, hence removing the need for rescanning, provided the calf veins have actually been visualized. We concluded that the only indications for a further duplex scan were a continued and significant clinical suspicion of DVT or an incomplete primary scan, i.e. incomplete visualization of the calf veins.

Mr Braithwaite also suggests that high-risk patients who have a normal scan, and low-risk patients who have an abnormal scan, should have venography. This assumes that venography is the gold standard for the investigation of DVT. Venography is invasive, uncomfortable, can cause DVT in up to 2 per cent of patients and misses the diagnosis in up to 10 per cent of patients[3]. We see no indication for the use of venography in the diagnosis of DVT, have demonstrated a strategy which avoids this and regard duplex scanning as the gold standard against which other tests should be measured.

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Letter 2

Sir

The notion of complete clearance of the venous system following thrombosis is appealing. Some natural history studies link early restoration of patency to improved valvular function and reduced postphlebitic sequelae. Reported complication rates for catheter-directed intrathrombus thrombolysis (CDITT) and surgical thrombectomy are remarkably low[1,2]. Both forms of intervention are more effective in those who have had recent thrombosis and some groups propose a week as the cut-off point[2]. Patients who have significant comorbidity, reduced activity, shortened life expectancy and bleeding complications are precluded from intervention. The degree of selection can be gauged from a reported series of 225 patients in which the intervention rate was less than 25 per cent[3].

The proponents of aggressive treatment of iliofemoral thrombosis agree that CDITT should be attempted first, with thrombectomy reserved for those with either contraindications to, or failure of, thrombolysis. Compared to anticoagulation alone CDITT results in improved early and late vein patency. Surgical thrombectomy achieves even better early and comparable late patency results, especially when combined with an arteriovenous fistula. Some would suggest surgical thrombectomy as the treatment of choice.

We urge caution in embracing these new forms of treatment. Available clinical data supporting aggressive treatment are from observational studies undertaken in only a few centres with relatively small numbers of carefully selected patients and short periods of follow-up. Even these studies fail to show any significant improvement in clinical outcome.

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Conservative management of appendix mass in children (*Br J Surg* 2001; 88: 1539–42)

Letter 1

Sir

I agree with the authors that appendicectomy in the presence of an appendix mass is technically more difficult. However, over half the children in this study were diagnosed by examination under anaesthesia. In my experience the presence of a mass detected in this way is not an accurate method of determining the difficulty of subsequent surgery. The results of this paper suggest that conservative management has a significant failure rate, requiring delayed emergency appendicectomy with an associated high complication rate and prolonged hospital stay. Children who underwent immediate appendicectomy had a shorter length of stay and low complication rate. The authors implied this was the consequence of a consultant surgeon performing the procedure. If this is the case then in current practice, the absence of a consultant surgeon out of hours is no longer an acceptable excuse for not operating on these children.

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Letter 2

Sir

Of the 346 children who were treated conservatively, 27 developed an appendix abscess and were treated with incision, drainage and subsequent appendicectomy. The median length of stay in this group was 17 (range 10–44) days. Conservative management includes evaluation of the mass using ultrasonography or computed tomography to distinguish abscess from phlegmon and then percutaneous drainage of the abscess[1,2], thus avoiding an operation and shortening the length of stay dramatically. Bagi *et al.*[3] have shown the median hospital stay to be 9 (3–23) days after radiological drainage. Similarly, vanSonnenberg *et al.*[4] have shown an average of 8 days. We recommend that this method of conservative management be used in selected cases that do not respond to standard treatment.

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Management of thoracic duct injuries after oesophagectomy (*Br J Surg* 2001; 88: 1442–8)

Letter 1

Sir

Following the pioneer work of Collard *et al.*[1,2] we tried etilefrine in the management of chylous leak after oesophagectomy. Etilefrine, an alpha-adrenergic drug used in postural hypotension, causes smooth muscle contraction. As the main lymphatic vessels, including the thoracic duct, contain smooth muscles, etilefrine might influence chyle flow after thoracic duct injury. Our experience with three patients has been reported[3] and we have experience of three other patients. Chyle leakage was diagnosed because of high concentrations of lymphocytes and chylomicrons. Chyle output was over 300 ml/day for all and higher than 1 l/day in two patients. Using a 5-mg/h intravenous etilefrine infusion, we observed a significant chyle flow reduction within 2 days. In five patients, chyle flow resolution was obtained within 5 days. In one patient there was a decrease of the chyle flow but etilefrine was stopped because of interactions with other sympathicomimetic drugs used for postoperative cardiorespiratory failure. Surgical exploration disclosed a supernumerary thoracic duct which had not been clipped during the first surgical procedure. We agree with a 48–72-h test of conservative treatment, but since the etilefrine effect was observed within 2 days, we feel that this drug may be considered as a useful adjunct.

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Letter 2

Sir

We agree that prevention of thoracic duct injury at the time of oesophagectomy is of the upmost importance. As patients having an oesophagectomy are fasted prior to surgery the thoracic duct is empty and identification may be difficult. To alleviate this problem we routinely administer oral cream to all our patients 3 h before surgery to aid in the identification of the thoracic duct at initial resection[1]. The duct is then easily identified and protected or ligated before division as necessary. If any accessory or aberrant ducts are divided the chyle leak can be identified and the vessel ligated. Prior to the introduction of this technique our incidence of postoperative chylothorax was 12 of 158 oesophageal resections (7·5 per cent). Over the past 2 years 93 patients were given cream before oesophagectomy and there have been no postoperative chylothoraces. The patients tolerated the cream well and did not experience any adverse events. We would therefore advocate the routine oral administration of cream before all oesophageal resections in order to prevent postoperative chylothorax.

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Randomized clinical trial comparing the efficacy of two bandaging regimens in the treatment of venous leg ulcers (*Br J Surg* 2002; 89: 40–4)

Letter 1

Sir

Meyer *et al.* found no improvement in leg ulcer healing using higher-compression bandages (Tensopress™) compared with low compression (Elastocrepe™). The implication of their conclusion is that a high-quality elastic bandage confers no healing benefit.

The authors are entitled to describe their bandages as differing in stretch but not as differing in compression, since they produced no data on the sub-bandage pressures achieved. When bandages are applied in multiple layers caution should be adopted in attributing healing rates to the elastic properties of an individual layer. Our laboratory studies confirm that the pressure exerted by a bandage is substantially influenced by adjacent layers (unpublished data).

The authors refer to our trial[1] which showed better healing rates with an elastic multilayered system compared with a relatively inelastic multilayered system. A key difference is their use of paste bandaging which adheres to the leg and also to the overlying bandage, preventing movement. They were therefore comparing two cohesive non-elastic systems. In such a system any difference in compression, or in skin damage, such as they reported, would be likely to depend on the force with which the bandage was applied rather than its elasticity. The debate as to the respective benefits of elastic *versus* non-elastic regimens remains unresolved.

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1 Callam MJ, Harper DR, Dale JJ, Brown D, Gibson B, Prescott RJ. Lothian and Forth Valley Leg Ulcer Healing Trial Part 1: elastic *versus* non-elastic bandaging in the treatment of chronic leg ulceration. *Phlebology* 1992; **7**: 136–41.

Authors' reply

Sir

We do have data on the sub-bandage pressure achieved by the Tensopress™ and Elastocrepe™ systems that were used, confirming that the Tensopress™ system exerts higher pressure[1]. It was found that the Tensopress™ regimen gave mean initial ankle pressures of 45 mmHg and the Elastocrepe™ regimen 24 mmHg using the Borgnis medical stocking test apparatus. We also state in our article that our bandaging systems were different from those used in the Edinburgh study. The debate between elastic and non-elastic systems may be further influenced by the three-layer paste *versus* four-layer bandaging study that we hope to publish shortly.

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1 Northeast ADR, Layer GT, Wilson NM, Browse NL, Burnand KG. Increased compression expedites venous ulcer healing. *The Venous Forum Annual Meeting*, 1990 (Abstract).

Diagnosis and management of a mediastinal leak following radical oesophagectomy (*Br J Surg* 2001; 88: 1346–51)

Sir

We read with interest this report from a single centre of the experience of a leak rate of 19 of 291 patients (6·5 per cent), six of 19 (32 per cent) of whom subsequently died. Given the public scrutiny of surgical complications and discussion concerning the size of institutions that should conduct oesophageal surgery, we wish to highlight the recent population-based audit of 3293 oesophageal and gastric cancers in Scotland[1].

Over a 2-year interval (to September 1999) the Scottish Audit of Gastric and Oesophageal Cancer (SAGOC) prospectively recorded data on all such cancers diagnosed in Scotland (with a minimum of a 1-year follow-up). Of the 1302 oesophageal, oesophagogastric junction and gastric cancers that had surgery, clinically detected leaks were recorded in 84 patients (6·5 per cent). For oesophageal cancer resections the leak rate was 37 of 457 (8·1 per cent);

17 patients (46 per cent of those with leaks) subsequently died. For oesophagogastric junction cancer 13 of 206 (6·3 per cent) patients had a leak and six died (46 per cent of those with leaks).

In SAGOC, however, the 663 oesophageal and gastro-oesophageal resections were performed in 30 hospitals of all sizes (96 per cent were performed by consultants). Detailed statistical analysis failed to show any case-load effect by hospital size. These data suggest that the model of managed clinical networks of hospitals may be as safe as the proposed 'superunits' when judged against surgical criteria such as postoperative leaks. The network model has the added advantage of local access for patients and maintains multidisciplinary clinical skills for upper gastrointestinal emergencies.

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[1] The Scottish Executive. *The Scottish Audit of Gastric and Oesophageal Cancer 1997–1999*. Edinburgh: The Scottish Executive, 2002.

Authors' reply

Sir

Our study included all causes of mediastinal leakage after oesophagectomy, with a mortality following true anastomotic leakage in two of 13 patients (15 per cent), leading to the death of less than 1 per cent of all patients in the series. This compares favourably with the Scottish Audit of Gastric and Oesophageal Cancer (SAGOC) data where 46 per cent of patients with a leak after oesophageal resection died, accounting for 3·7 per cent of all patients. Anastomotic leakage is an important measure of surgical technique but it is inappropriate to use this alone to support the model of managed clinical networks in Scottish hospitals. The management of these complex patients requires a multidisciplinary approach and more important outcome measures are the risk-adjusted overall in-hospital mortality and stage-specific survival.

Based on all the current evidence, the government has recently published guidelines for improving the outcome of upper gastrointestinal cancer in England and Wales[1]. It is recommended that surgical resection for oesophagogastric cancer is performed within cancer centres containing all necessary multidisciplinary services, and serving a population of approximately 1 million. When the SAGOC data are published in full in the future, they may shed light on whether Scotland will follow a similar plan for provision of services to England and Wales.

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[1] Department of Health. *Guidance on Commissioning Cancer Services: Improving Outcomes in Upper Gastrointestinal Cancers*. London: Department of Health, 2001.

Underpowered studies (*Br J Surg* 2002; 89: 129–31)

Sir

The authors' arguments in favour of small underpowered studies are compelling and informative; however, counter arguments have not been discussed fully. The debate is actually an extension of the comparison of 'meta-analysis of smaller trials *versus* large trials'[1,2]. The suggestion that meta-analysis of the smaller underpowered trials is a solution to the problems of underpowered studies is controversial. Numerous problems exist with meta-analyses: publication bias, covert duplicate of data, etc.[3]. Meta-analyses can also be misleading[4]. A recent study that compared large randomized trials to previous meta-analyses found that the latter would have led to the adoption of an ineffective treatment in 32 per cent of patients and rejection of a useful treatment in 33 per cent[2]. Is prevention (the performance of good quality adequately-powered studies) not better than cure (meta-analyses of several small underpowered inconclusive studies)?

The authors themselves agree that small trials are 'on average lower quality'. Quality depends on proper planning and determination of power and sample sizes. Clinically important differences between interventions can be determined by an understanding of the outcome measures, actual benefits/side-effects to patients and/or the healthcare system, and the cost-effectiveness of the interventions under scrutiny. Studies with low power waste valuable time and resources and delay the initiation of further studies with adequate power to reject the null hypothesis; for these reasons they can be considered unethical[5]. With due respect to the authors' knowledge and expertise in the field, apart from the accuracy and good quality of the clinical trials, prior sample size estimations and planning to obtain an adequately powered study should still be an important criteria for editors to consider before publication.

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1 Cappelleri JC, Ioannidis JPA, Schmid CH, de Ferranti SD, Aubert M, Chalmers TC *et al.* Large trials *versus* meta-analysis of smaller trials: how do their results compare? *JAMA* 1996; **276:** 1332–8.
2 LeLorier J, Gregoire G, Benhaddad A, Lapierre J, Derderian F. Discrepancies between meta-analyses and subsequent large randomized, controlled trials. *N Engl J Med* 1997; **337:** 536–42.
3 Naylor CD. Meta-analysis and the meta-epidemiology of clinical research. *BMJ* 1997; **315:** 617–19.
4 Egger M, Davey Smith G. Misleading meta-analysis. *BMJ* 1995; **310:** 752–4.
5 Altman DG. Statistics and ethics in medical research. III. How large a sample? *BMJ* 1980; **281:** 1336–8.

Randomized clinical trial of Ligasure™ *versus* open haemorrhoidectomy (*Br J Surg* 2002; 89: 154–7)

Sir

The authors were overzealous in calculating the power of the study, which resulted in inadequate numbers in each group and made valid conclusions impossible. I agree that this technique is easy to learn and takes less time to perform. It would be nice to know how many patients in each group had previous injection sclerotherapy. Adherence of sphincter muscle to the haemorrhoids is common, which in a diathermy haemorrhoidectomy[1] would be dissected away, but in the Ligasure™ group could be picked up and result in a sphincter injury. The authors should be encouraged to assess the Ligasure™ patients with histology of the haemorrhoids, endoanal ultrasonography and anal manometry to assess the anal sphincters. Though it appears there was no incontinence in either group, it would be good to know if there were any anatomical or pressure changes in the Ligasure™ group that may manifest later. Long-term results of the Ligasure™ technique will be the yardstick used to measure its ability to cure haemorrhoids[2].

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1 Seow-Choen F, Ho YH, Ang HG, Goh HS. Prospective, randomized trial comparing pain and clinical function after conventional scissors excision/ligation *versus* diathermy excision without ligation for symptomatic prolapsed hemorrhoids. *Dis Colon Rectum* 1992; **35:** 1165–9.
2 Cheetham MJ, Philips RKS. Evidence-based practice in haemorrhoidectomy. *Colorectal Disease* 2001; **3:** 126–34.

Upper Gastrointestinal Surgery: A Companion to Specialist Surgical Practice. 2nd Ed.

S. M. Griffin and S. A. Raimes (eds)

250 × 190 mm Pp 474 Illustrated. 2001 London. WB Saunders. £54·95

This book has been very carefully composed. It covers all the topics relevant to upper gastrointestinal surgery. It highlights the topical issues and unanswered questions in two different ways: in the text by separate subsections, and by adding a short summary to key references. This makes the volume very approachable and suitable for a quick update of knowledge when confronted with a difficult clinical problem. The list of references does not contain the most up-to-date literature, but this is not a specific shortcoming of the book. The quality of the illustrations, apart from the line drawings, tables and flowcharts, shows some variation. The radiographs and photographs of operative procedures, for example in chapters 10 and 14, are mediocre; it seems as though something has gone wrong with the printing process. In several other chapters, such as chapter 3, similar illustrations are of good quality. The volume guides the reader through the field of upper gastrointestinal surgery and fully meets the demands of 'a companion' for surgical trainees and surgeons. Overall, it can be highly recommended.

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Sabiston Textbook of Surgery: The Biological Basis of Modern Surgical Practice. 16th Ed.

C. M. Townsend, R. D. Beauchamp, B. M. Evers and K. L. Mattox (eds)

286 × 220 mm. Pp. 1750 Illustrated 2001. Philadelphia, Pennsylvania. WB Saunders. $129·00.

"It is astonishing with how little reading a doctor can practice medicine, but it is not astonishing how badly he may do it." Sir William Osler.

This vintage 'oslerism' headed the preface of my well thumbed 10th edition. First published in 1936, this work seeks to provide the most current and comprehensive information on surgical science and treatment. Generations of budding surgeons have cut their teeth using this book as the mainstay of their libraries. Despite the availability of several other tomes from both sides of the Atlantic which attract the same readership, the *Textbook of Surgery*, now in its 16th edition, has flourished at the top end of a fiercely competitive market among the flagship texts in general surgery.

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Scientific Surgery

The *BJS* is committed to the practice of surgery based on scientific evidence. Each month we will publish a list of randomized trials and meta-analyses collated from English-language publications. A collection of all these papers will be available in the Scientific Surgery archive which will be held on the *BJS* website together with links to free Medline sites where the full papers can be accessed (see below for details). If you have recently published a randomized trial that has not been featured in Scientific Surgery, the Editors would be pleased to receive a reprint and consider its inclusion.

Lindholt JS, Juul S, Fasting H, Henneberg EW. Hospital costs and benefits of screening for abdominal aortic aneurysms. Results from a randomised population screening trial. *Eur J Vasc Endovasc Surg* 2002; **23:** 55–60.

Over 12 000 men were invited; 76 per cent attended. Six men in the screened group and 19 in the control group died from an aneurysm after a mean of 5 years of observation.

Danielsson G, Jungbeck C, Peterson K, Norgren L. A randomised controlled trial of micronized purified flavonoid fraction *versus* placebo in patients with chronic venous disease. *Eur J Vasc Endovasc Surg* 2002; **23:** 73–6.

The flavonoid fraction did not improve any symptoms except night cramps in this study of 101 patients with chronic venous insufficiency. Ultrasonographic reflux was reduced in the treatment group, but foot volumetry was unchanged.

Dey P, Bundred N, Gibbs A, Hopwood P, Baildam A, Boggis C *et al.* Costs and benefits of a one stop clinic compared with a dedicated breast clinic: randomised controlled trial. *BMJ* 2002; **324:** 507–10.

Some 670 women aged 35 years or over with a breast lump were randomized. Anxiety levels were reduced 24 h after a one-stop clinic visit, but not 3 weeks or 3 months later; the cost was an extra £32 per woman.

Bellantone R, Boscherini M, Lombardi CP, Bossola M, Rubino F, De Crea C *et al.* Is the identification of the external branch of the superior laryngeal nerve mandatory in thyroid operation? Results of a prospective randomized study. *Surgery* 2001; **130:** 1055–9.

This study included 289 consecutive patients who had careful ligation of the upper pole thyroid vessels with, or without, identification of the nerve. Detailed investigation of voice patterns after operation showed no changes and no difference between the groups.

Jorgensen OD, Kronborg O, Fenger C. A randomised study of screening for colorectal cancer using faecal occult blood testing: results after 13 years and seven biennial screening rounds. *Gut* 2002; **50:** 29–32.

This study from Denmark included over 60 000 subjects. Mortality from colorectal cancer was significantly reduced in the screened group: relative risk 0·82 (95 per cent confidence interval 0·69 to 0·97).

Gencosmanoglu R, Sad O, Koc D, Inceoglu R. Hemorrhoidectomy: open or closed technique? A prospective, randomized clinical trial. *Dis Colon Rectum* 2002; **45:** 70–5.

Although the open method took longer to heal, analgesic requirement and morbidity rates were lower and the operation was quicker in this study of 80 operations. Duration of hospital stay and time off work were similar.

Ho Y-H, Yu S, Ang E-S, Seow-Choen F, Sundram F. Small colonic J-pouch improves colonic retention of liquids – randomized, controlled trial with scintigraphy. *Dis Colon Rectum* 2002; **45:** 76–82.

Twelve patients were studied after having low anterior resection. The scintigrams suggested there was better retention of liquid stool in the distal colon in patients who had a pouch, leading to reduced frequency of defaecation.

Vinson-Bonnet B, Coltat JC, Fingerhut A, Bonnet F. Local infiltration with ropivacaine improves immediate postoperative pain control after haemorrhoidal surgery. *Dis Colon Rectum* 2002; **45:** 104–8.

Early postoperative pain was significantly improved in this trial that included 34 procedures. Visual analogue pain scores were improved and morphine requirement was less.

Miccoli P, Berti P, Raffaeli M, Materazzi G, Baldacci S, Rossi G. Comparison between minimally invasive video-assisted thyroidectomy and conventional thyroidectomy: a prospective randomized study. *Surgery* 2001; **130:** 1039–43.

Some 49 procedures were studied. Video-assisted surgery took longer (66 *versus* 45 min, $P = 0{\cdot}001$), but was less painful and had better cosmetic results. Outcome and complications were similar.

Koussidis GA, Koussidis A. Preoperative bowel preparation with meglumine and sodium diatrizoate (Gastrografin): a prospective randomised comparison *Eur J Surg* 2001; **167:** 899–902.

Gastrografin was better tolerated in this study which included 58 patients being prepared for elective colorectal procedures. Both preparations were equally effective: over 90 per cent in each group had a clean colon at operation and the remainder had only slight residue.

The *BJS* website can be found at *www.bjs.co.uk*. In addition to details of current and previous articles featured in Scientific Surgery, the website contains details of the Editorial Team and Board, instructions to prospective authors, links to other sites (such as The Cochrane Library) and forthcoming surgical events. The Editors welcome comments and suggestions about the website, either in writing or through the website address.

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Type the references with double spacing in the Vancouver style (see **Preparation of manuscripts**). Reference to abstracts and personal communications is discouraged. Reference to unpublished communications will not be accepted.

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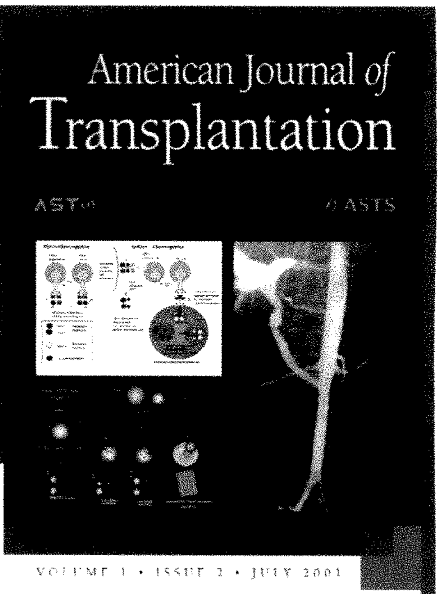
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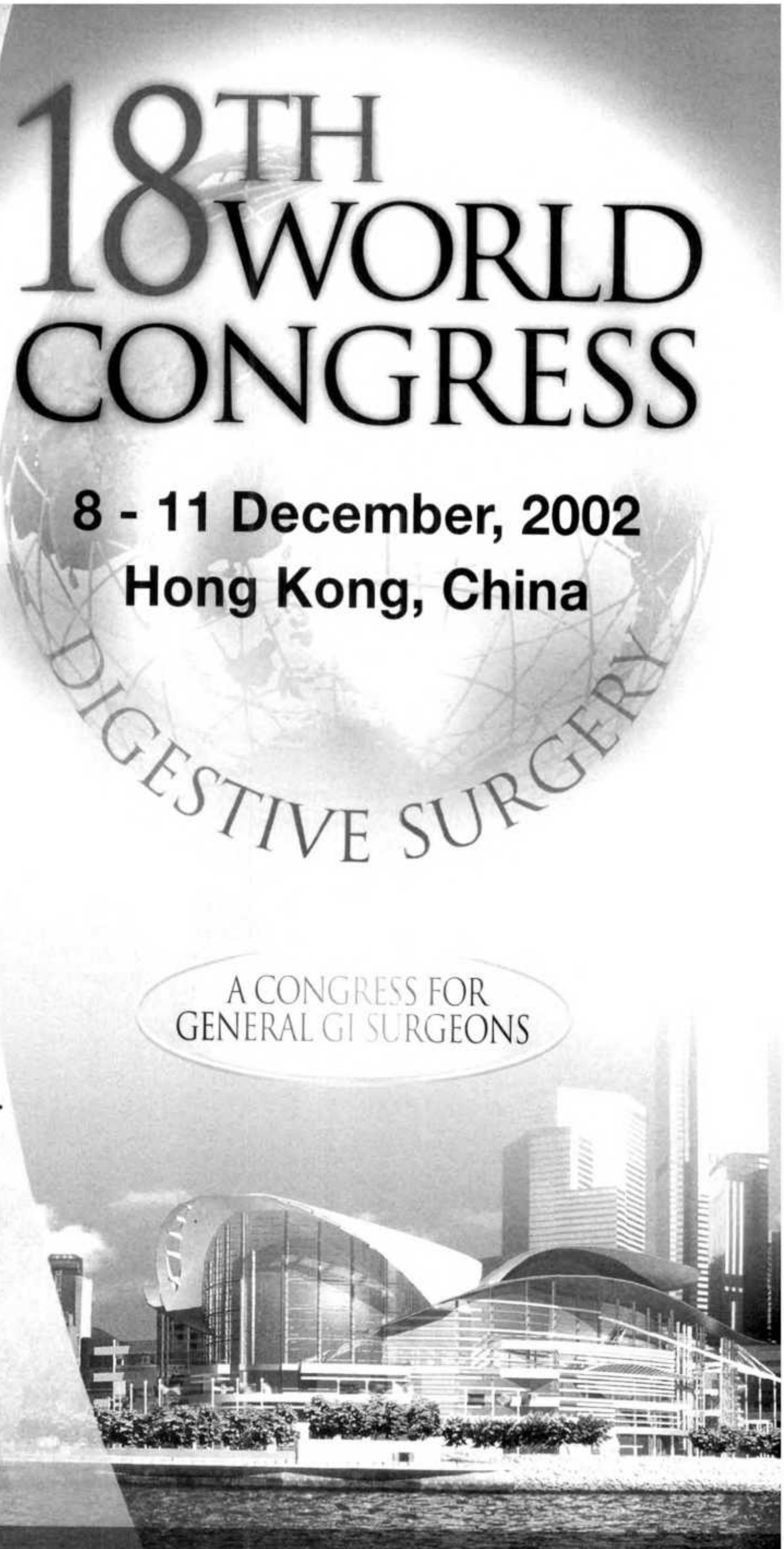

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Leading article

Contrast-enhanced magnetic resonance angiography

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The first magnetic resonance angiography (MRA) techniques were flow-based and these methods are hampered by troublesome artefacts, particularly where laminar flow is disrupted at stenoses or is slow and disordered as in aneurysms. Limited volume coverage, poor signal from deep body structures and long imaging times during which patient movement further degrades images limited clinical acceptance outwith neurovascular applications. These limitations have been overcome with the advent of gadolinium contrast-enhanced MRA (CE-MRA) which has propelled the technique to the forefront of diagnostic vascular imaging.

Thoracic aorta, arch branches and carotid arteries

MRA is well suited to the assessment of coarctation, especially in follow-up after repair when there is suspicion of restenosis or other complication. Thoracic aneurysms (*Fig. 1*), great vessel disease and dissection are also all well assessed[1]. This can be combined with evaluation of aortic valve incompetence and cardiac function at the same examination.

Assessment of the carotid arteries (*Fig. 2*) by MRA is gaining in importance in validating the findings of duplex ultrasonography without the risk of invasive angiography. MRA is especially useful when duplex findings are equivocal and when significant tandem disease at the great vessel origin or carotid siphon is suspected. While imaging access to the carotid arteries is restricted with ultrasonography, MRA can provide a comprehensive overview from the aortic arch to the intracranial circulation[2].

There is a trade-off to be made between fast imaging and high spatial resolution and/or high contrast. These conflicting demands are well illustrated in carotid imaging, where high spatial resolution is critical for accurately defining disease while venous enhancement must be avoided by rapid imaging (circulation time from carotid arteries to jugular veins is in the order of just 8 s). Newer optimized pulse sequences and improved hardware are successfully addressing these issues[3].

Renal arteries

MRA evaluation of the renal arteries for occlusive disease is a major application and the early promise as a non-invasive screening technique is now coming to fruition. Gadolinium agents have negligible nephrotoxicity, an important advantage when imaging patients with renal insufficiency. Renal CE-MRA is also indicated for the assessment of vascular anatomy in living related donors for transplantation, avoiding the use of invasive angiography with ionizing radiation[4]. An initial morphological study of the retroperitoneum is useful in depicting renal anatomy, and magnetic resonance imaging has been shown to be more accurate in the assessment of renal size and detection of pathology than ultrasonography. The problem of renal transplant artery stenosis is also well suited to MRA assessment.

The use of contrast also brings the potential with dynamic serial imaging to provide renal perfusion/functional information at the same time as the angiogram. New techniques such as parallel imaging are permitting 'time-resolved' acquisitions that can track bolus passage while maintaining spatial resolution, with the potential to acquire angiographic and perfusion imaging at the same time[5].

Splanchnic arterial branches and portal venous system

The coeliac and superior mesenteric artery origins are well defined with CE-MRA making it ideal for assessing suspected mesenteric angina[6]. While studies have shown MRA to be accurate in identifying major visceral vessel variations, there is only limited experience in the evaluation of these vessels for encasement (e.g. staging pancreatic carcinoma). In all enhanced splanchnic studies, venous phase imaging is acquired routinely immediately after the arterial study to show the portal venous system (*Fig 3*) and hepatic veins.

MRA has been shown to be the most sensitive and accurate imaging modality for the assessment of variceal pathways and portosystemic shunts in portal hypertension, as well as for the assessment of portal venous patency before portosystemic shunt creation and liver transplantation[7]. As CE-MRA provides both portal and hepatic venous anatomy, it can be used as part of comprehensive liver evaluation before transjugular intrahepatic portosystemic shunt to produce a spatial map of the venous anatomy to plan the formation of a suitable shunt tract (in what may be a distorted liver parenchyma) and exclude an extrahepatic portal bifurcation when the liver is shrunken.

Abdominal aorta, iliac and lower-limb arteries

The abdominal aorta may be studied for evaluation of aneurysmal disease, stenosis, inflammatory change and dissection, with an accuracy comparable to that of digital subtraction angiography (DSA) and computed tomography (CT) angiography. CE-MRA is particularly indicated for the assessment of occlusive aortoiliac disease (*Fig. 4*), especially where there are poor femoral pulses, grafts (extra-anatomic, occluded or complicated by false aneurysms) or groin sepsis (when invasive access may be problematic). The initial problem with CE-MRA in the lower limbs was one of length of coverage; this has now been overcome with systems utilizing a stepping table technique similar to those now common in conventional angiography suites, with analogous masking methods. Using these systems, evaluation of the aortoiliac vessels and the whole lower-limb arterial run-off has been reduced to a short outpatient scan appointment, requiring no special preparation other than an intravenous injection of non-nephrotoxic contrast agent. The accuracy of these techniques compared with conventional DSA has been well validated in several studies[8].

Systemic veins and pulmonary vasculature

Although MRA is accurate in the assessment of venous disorders, it is generally reserved for regions not easily amenable to duplex ultrasonography, such as the thoracic veins, pelvic veins and vena cava. Recent work has also shown a high accuracy in the detection of calf deep vein thrombosis for which ultrasonography may be specific but

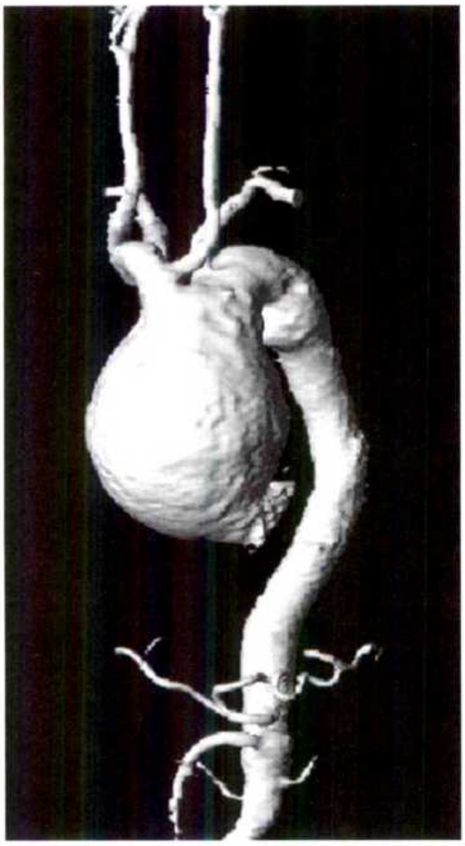


Fig. 1 Surface shaded rendering of thoracic aorta showing huge ascending aortic aneurysm and relation to great vessels

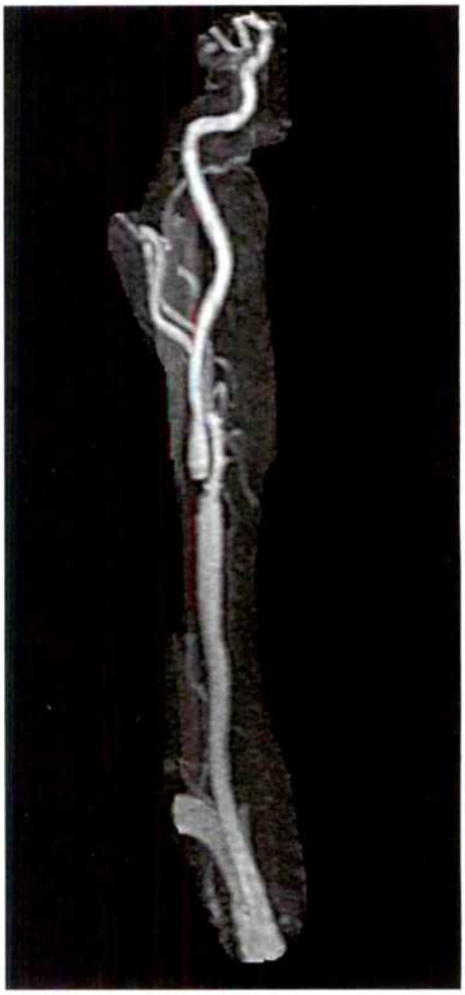


Fig. 2 Carotid magnetic resonance angiography using phased array coil; detailed view of right carotid from origin to circle of Willis showing internal carotid origin stenosis

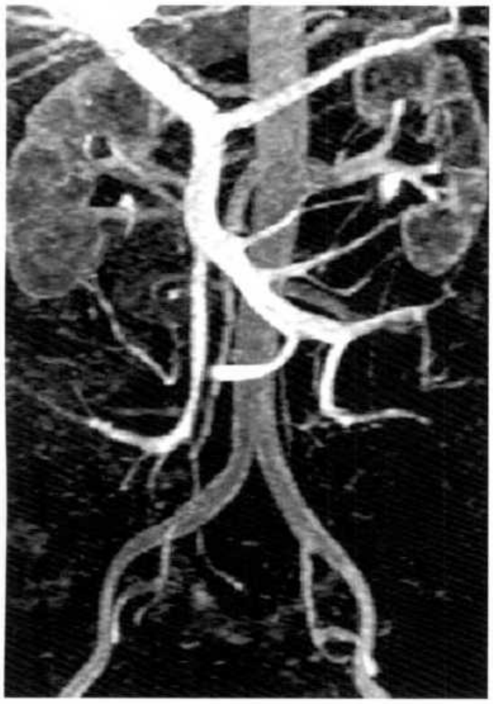


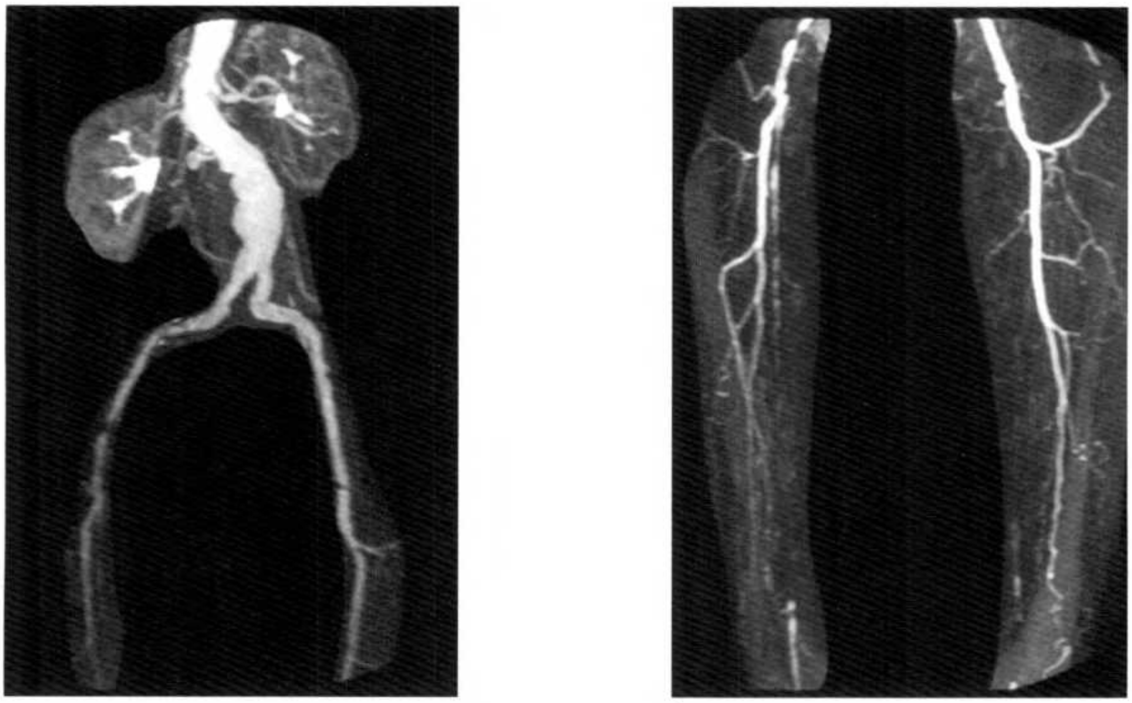
Fig. 3 Normal portal venous magnetic resonance angiography; note high contrast obtained with background context of related arterial anatomy

insensitive; examination is often hampered in the swollen or ulcerated limb.

Pulmonary MRA is technically challenging if rapid enough imaging to separate pulmonary arterial from pulmonary venous phases is sought, although this is not an absolute requirement for diagnosis[9]. The advantage of MRA for the investigation of pulmonary embolism is that it can provide perfusion information as well as direct vessel visualization. While spatial resolution is currently inferior to that of helical CT techniques, MRA has a role in the young, in pregnancy, in monitoring of therapeutic results and when central vein visualization is required, such as in patients with central venous lines.

Limitations, pitfalls and economics

MRA is limited by the same factors as any other magnetic resonance imaging. It is contraindicated in pacemaker-dependent patients and in those with internal defibrillators (and various other implanted systems). Local metalwork, such as hip prostheses and surgical clips, causes artefacts that may limit imaging; vascular stents are particularly problematic. Many stents currently implanted are stainless steel and their interior cannot be visualized. However, some



a Aorta, iliac

b Femoral artery

Fig. 4 a and **b** Two stations from peripheral magnetic resonance angiography study showing aneurysmal aorta, stenotic iliac disease and occlusive superficial femoral artery disease stent materials, such as nitinol, are more MRA friendly and can be imaged successfully, particularly those of larger calibre. The other main limitation is that of spatial resolution which does not yet match that of conventional radiographic angiography. Vascular intervention using MRA is theoretically possible and is currently an area of research; several studies have shown the practicality of angioplasty in animal models, although clinical reality is some years off[10].

The large number of images produced means that interpretation of MRA must be performed on a workstation. Maximum intensity projection (MIP) images provide overview anatomy in a familiar 'angiographic' format, but reliance on these without reference to the base images may lead to misdiagnosis. MIP reformation is a valuable tool, but the quality of image is dependent on the care taken during editing and the radiologist reporting must be aware of all the data for a study. On occasion, surface shaded display (SSD) may prove helpful for particularly tortuous anatomy as it helps orientation. SSD is, however, even more prone to masking or exaggerating pathology than MIP.

Economically MRA is attractive, certainly in comparison to conventional angiography for which capital and staff costs are high. In this institution analysis has shown MRA, at £150 basic National Health Service cost, to be 44 per cent cheaper than conventional angiography (71 per cent cheaper when bed costs are included), allowing significant savings despite a rising diagnostic workload. Departmental data since the introduction of MRA 4 years ago show a 42 per cent reduction in conventional diagnostic angiography while interventional vascular procedure rates have remained steady. Although the number of patients overall undergoing angiographic diagnosis (MRA plus conventional angiography) has increased by 35 per cent (mainly for renovascular assessment), there has been a theoretical saving of about £100 000 per annum while waiting times have halved.

Conclusion

MRA is a rapidly advancing and exciting modality that, where available, is becoming the preferred first-choice vascular imaging procedure in many diagnostic situations. With advances in hardware and software, the only barriers to its widespread routine use will be machine availability and radiological expertise.

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Leading article

Patient volumes and complications in thyroid surgery

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There are three well recognized complications of thyroid surgery, recurrent laryngeal nerve dysfunction, hypoparathyroidism and postoperative bleeding; their incidence is a sensitive measure of the quality of the thyroidectomies being performed. Reported complication rates vary widely, from 0 to 14 per cent for recurrent laryngeal nerve injury, 1–11 per cent for permanent hypoparathyroidism, and 0–1 per cent for postoperative bleeding[1]. Differing complication rates may reflect variation in surgical experience, but other variables are related to outcome as well as the simple number of operations a surgeon performs.

The association between the volume of an operation performed and outcome has important implications for enhancing quality and reducing the cost of healthcare. Investigation of volume–outcome relationships is not a new concept. Luft *et al.*[2] in 1979 addressed the issue of regionalization of operations based on surgical volumes and mortality. Since then, many studies have evaluated the relationship between patient volume and outcome for a number of surgical procedures and for treatment of various medical conditions. It has been suggested that providers who treat larger volumes of patients have better outcomes[3–5]. The business community in the USA is actively acquiring information on quality of care. The Leapfrog Group, a large coalition of major corporations, is using such information to determine which facilities will be used to provide healthcare for their employees[4].

The Institute of Medicine found 88 volume–outcome studies that met its critical review for scientific merit and 77 per cent of them demonstrated a significant association between patient volume and outcome[3]. Volume–outcome studies have demonstrated an association between higher volume and lower in-hospital mortality rates for various operations, including pancreaticoduodenectomy, oesophagectomy, gastrectomy, colectomy, lung lobectomy, abdominal aortic aneurysm repair, carotid endarterectomy and coronary artery bypass grafting[4,5]. The National Cancer Registry Board considered that the volume–outcome data for treatment of patients with oesophageal and pancreatic cancer were so compelling that it has recommended the referral of such patients to high-volume hospitals recognized as centres of excellence.

The relationship between volume of operations performed and outcome has also been examined for thyroid surgery, but not to the extent of some other operations. Sosa *et al.*[6] demonstrated a significant inverse relationship between the volume of thyroidectomies performed by individual surgeons in Maryland and complication rates, postoperative length of stay and hospital charges. Surgeons performing 100 or more thyroidectomies over a 6-year period had the lowest complication rate, the shortest hospital stay and the lowest hospital charges compared with surgeons performing 30–100 cases, 10–29 cases and between one and nine cases. The high-volume surgeons had one-third fewer complications from thyroidectomy performed for benign disease, and two-thirds fewer complications from thyroidectomy performed for cancer, than surgeons who performed between one and nine thyroidectomies during the 6-year period. The authors make a case for referring to high-volume surgeons those patients with known or suspected thyroid cancer or for whom near-total or total thyroidectomy is considered the procedure of choice.

Lamade and colleagues[7] reported nearly twice as high an incidence of early recurrent laryngeal nerve dysfunction for surgeons who had completed their training and were not specializing in thyroid surgery, compared with surgical trainees and established surgeons specializing in thyroid surgery. The lower incidence of complications for surgical trainees was probably the result of extensive supervision by more experienced operators. Mishra *et al.*[8] also reported similar incidences of recurrent laryngeal nerve injury and permanent hypoparathyroidism following total thyroidectomy performed by endocrine surgical trainees and by experienced (consultant) surgeons.

Other factors that are important in performing safe and effective thyroid surgery include a thorough understanding of normal neck anatomy and anatomical variations, and comprehensive training in the appropriate surgical techniques. It should be recognized that the incidence of recurrent laryngeal nerve injury increases with the size of the diseased thyroid and with the extent of thyroid resection[9]; when appropriate, total thyroidectomy should be avoided. Routine exposure of the recurrent laryngeal nerve throughout its entire course has been shown to reduce the rate of recurrent laryngeal nerve injury[9]. This should be a component of every thyroidectomy as it allows the surgeon to visualize and avoid injury to the nerve during thyroid resection. The operator should be aware of the possibility of a non-recurrent laryngeal nerve which comes off the vagus nerve in the neck in approximately 0·5 per cent of individuals, more often on the right side. Injury to the superior laryngeal nerve is avoided by ligation of the branches of the superior thyroid artery close to the capsule of the thyroid gland.

Hypoparathyroidism after thyroid surgery is prevented by preserving the parathyroid glands *in situ* and by autotransplanting parathyroid tissue that cannot be so preserved. *In situ* preservation of the parathyoid glands and their blood supply is accomplished by individual ligation of the branches of the inferior thyroid artery on the surface of the thyroid lobe and leaving the glands within their envelope of fat. When a gland cannot be preserved, a small piece should be submitted for frozen-section examination to confirm that the tissue is parathyroid. The remaining gland is then sliced into multiple sections and is autotransplanted into the sternocleidomastoid muscle. Preservation of the recurrent laryngeal nerves and the parathyroid glands by their identification is a better approach than using the technique of capsular dissection with the presumption of safety as advocated by some surgeons.

To reduce complications, general surgery training programmes need to provide an adequate operative experience in thyroid surgery. Harness *et al.*[10] previously evaluated the operative experience in the USA of general surgery residents in thyroid surgery, and concluded that general surgery residency training programmes do not give adequate exposure to such operations. The mean number of thyroidectomies performed as 'surgeon' by a graduating chief resident in 1994 was only 12·5 and the mode was 8, raising concern as to whether residents were receiving adequate experience in operating for thyroid cancer, substernal goitre and Graves' disease[10]. By 2001, the mean number of thyroidectomies had increased to only 15.

Training can be improved by having a competent endocrine surgeon on the faculty of all academic programmes to ensure that general surgery trainees obtain the requisite surgical skills to perform a safe and effective thyroidectomy. For programmes that routinely do not meet the minimum requirements for endocrine surgical procedures, special endocrine surgery rotations should be organized at other institutions. The structure of training programmes may need to be changed so that thyroidectomies are channelled to trainees planning careers in general surgery and away from those planning to be plastic, vascular or cardiovascular surgeons. For those interested in specializing in thyroid surgery, added training should be pursued to obtain more experience in performing total thyroidectomy, central and modified neck dissections, and reoperative neck surgery. Furthermore, it may be worthwhile to investigate a means of minimizing the temporary increase in complication rates that occurs during the maturation of surgeons immediately out of training. A new surgeon could be paired with a senior colleague who could be consulted for advice and who would act in a supervisory role for more difficult or extensive operations.

In summary, there are many variables that contribute to a successful outcome from thyroidectomy. Improving surgical training, encouraging additional fellowship training for those interested in specializing in thyroid surgery, providing consultation and supervision for new surgeons, and directing more difficult thyroidectomies to high-volume surgeons are a few steps that might be taken to improve outcome and enhance patient care. It is also important to continue to accumulate surgical outcome data, specifically for total thyroidectomy, modified neck dissection and reoperative thyroidectomy, to aid policy decisions about who should be performing such procedures.

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Genetic pathways involved in the progression of Barrett's metaplasia to adenocarcinoma

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Background: The prediction of which patients with Barrett's metaplasia will develop cancer is difficult. Better genetic characterization of the condition may aid clinicians in devising more effective management and follow-up strategies.

Methods: A review was undertaken of the accumulated genetic data relating to the progression of squamous epithelium to adenocarcinoma. The normal functions of a number of cancer-related genes are described and an explanation is given of how alterations in these genes interfere with normal cell processes and lead to cancer.

Results and conclusion: The main genetic alterations accompanying the progression through dysplasia to adenocarcinoma were collated from 135 papers. The principal genetic changes implicated are the loss of *p16* gene expression (by deletion or hypermethylation), the loss of *p53* expression (by mutation and deletion), the increase in cyclin D1 expression, the induction of aneuploidy and the losses of the *Rb*, *DCC* and *APC* chromosomal loci.

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Introduction

Norman Barrett is credited with the discovery in 1950 of metaplastic columnar epithelial cells lining the lower oesophagus[1]. He originally believed that the columnar lining was the result of a short oesophagus that drew stomach tissue up into the thoracic cavity[2]. Barrett's oesophagus is now recognized as a metaplastic condition in which squamous epithelial cells are replaced by intestinal-type columnar epithelial cells as a consequence of gastro-oesophageal reflux disease. Barrett's oesophagus is defined as a columnar-lined oesophagus with intestinal metaplasia on biopsy, accompanied by the existence of goblet cells, and Barrett's metaplasia represents the first step in the pathway to oesophageal adenocarcinoma. The metaplastic oesophagus subsequently progresses through low-grade dysplasia (LGD) and high-grade dysplasia (HGD) to adenocarcinoma. Currently, despite recent advances in laser ablation techniques and photodynamic therapy, no treatment is available that can reverse the metaplastic state[3] or remove the risk of progression to adenocarcinoma[4]. The risk of progression to higher grades of dysplasia and adenocarcinoma cannot be predicted accurately, and so patients are entered into endoscopic surveillance programmes, placing a significant burden on health services[5].

Role of reflux in Barrett's metaplasia

Reflux induces damage to squamous epithelial cells and causes stem cell proliferation, resulting in the replacement of squamous cells with columnar cells[6]. This cell conversion may initially progress through the appearance of simple columnar cells before specialized intestinal cells are evident. The process of metaplasia may be enhanced through the folding of the basal epithelium, which may physically position stem cells closer to the refluxate[3]. For a review of the potential mechanisms of metaplastic transformation, see Jankowski *et al.*[7]. Metaplastic intestinal-type columnar cells may be more resistant to the refluxate because of their mucin-producing status, and so may be positively selected for by further refluxate exposure[8]. As well as gastro-oesophageal reflux, severe mucosal damage may also be caused by the components of duodenal juice during duodenogastro-oesophageal reflux. Duodenal reflux may therefore further promote neoplastic development in oesophageal tissue[9,10].

Barrett's metaplasia

Metaplastic Barrett's segments may vary in length; indeed, segment length represents an important factor in disease progression and metaplasia may be divided into short-segment (3 cm or less) or long-segment (more than 3 cm) forms. Ultra-short-segment metaplasia, also known as cardiac intestinal metaplasia, describes an endoscopically normal gastro-oesophageal junction with histologically detectable goblet cells. Such ultra-short-segment lesions may represent a separate disease[2,11], perhaps linked to infection of the stomach with *Helicobacter pylori*[12–15]. The risk of developing adenocarcinoma is proportional to the segment length[16]. Long-segment Barrett's metaplasia represents the most important risk factor for progression to adenocarcinoma[17]; short-segment disease is associated with a lesser risk[18].

Dysplasia

Surveillance of metaplasia requires regular endoscopic examination, with 2-cm quadrantic biopsies. At present, histological detection of dysplasia is the principal means of assessing the risk of cancer progression[19]. Biopsies are currently categorized as negative for dysplasia, indefinite for dysplasia (where there is difficulty in distinguishing between dysplasia and regenerative change), LGD and HGD. While good interobserver correlation is obtained with HGD, there exists considerable variation when interpreting LGD and the indefinite category. HGD may be further subdivided into focal or diffuse types. Diffuse HGD has recently been shown to carry a higher risk of progression to adenocarcinoma[20]. Some 50 per cent of patients with HGD may already have an adjacent adenocarcinoma[3]. It has also been shown that the surgical removal of HGD tissue leads to 5-year survival rates greater than 80 per cent[2], and so HGD appears to be highly predictive of cancer development[21,22]. However, the rate of development of HGD from metaplasia is not known.

Adenocarcinoma

Over the past 30 years, the rise in the incidence of Barrett's adenocarcinoma has been greater than that for any other malignancy in the USA[6,23] and has shown an eightfold increase in the UK over the same period[2]. Currently, in these Western countries, adenocarcinoma is ranked as the fifth most prevalent cancer[24]. This rise has been linked to a corresponding increase in the incidence of Barrett's metaplasia[25]. The survival rates from oesophageal cancer are poor[26]; indeed, the mortality rates from adenocarcinoma often reflect the incidence rates[27,28]. Thus Barrett's associated adenocarcinoma needs to be diagnosed and treated early if any improvement in long-term prognosis is to be achieved.

Risk factors for Barrett's metaplasia

Barrett's metaplasia is most often diagnosed in caucasian men[29]; it is linked to cigarette smoking[16] and obesity[25,30]. It occurs relatively more frequently in patients with a low intake of fruit and vegetables[31], fibre[32] and selenium[33]. The risk of developing oesophageal adenocarcinoma is 30–125-fold higher in patients who already have Barrett's metaplasia[34]. Given that 15–30 per cent of people experience reflux regularly[3,25], and that 5–10 per cent of them are likely to develop metaplasia[2,25], up to 3 per cent of the Western population may have Barrett's metaplasia. The scale of the problem is highlighted by the fact that 7000 new cases of oesophageal adenocarcinoma are diagnosed annually in the UK[35].

Only 5–10 per cent of patients with reflux develop metaplasia[3], demonstrating the influence of host genetic factors on the acquisition of Barrett's metaplasia. It is known that chronic reflux is a feature in only 60 per cent of those with metaplasia[3]; some patients develop adenocarcinoma without a history of acid reflux[25] and without metaplasia[35]. Other important factors seem to be crucial in the development of metaplasia and adenocarcinoma; these may include exposure of the oesophagus to nitrosamines[31], such as *N*-nitrosodiethylamine and *N*-nitrosodimethylamine[36]. Conversely, the presence of *H. pylori* has been suggested as a protective factor, because those with metaplasia tend not to have CagA$^+$ strains of this organism[37–41]. This protective effect may be related to *H. pylori*-induced gastric atrophy, which may reduce stomach acid production.

Problems associated with the identification and staging of Barrett's metaplasia

Only a small proportion of patients with Barrett's metaplasia develop adenocarcinoma and so routine endoscopic surveillance of all such patients may not be cost-effective. Variation between clinicians exists in endoscopic surveillance regimens[42] and there are large numbers of false-positive diagnoses of metaplasia[43]. Interobserver variation is also a problem in the grading of progression of disease[44]. Molecular approaches are urgently required to pinpoint diagnostic markers to permit accurate identification of individuals at greater risk of progression to adenocarcinoma.

Genetic alterations during the progression of Barrett's metaplasia to adenocarcinoma

A large number of genetic events are important in the metaplasia–dysplasia–adenocarcinoma (M-D-A) sequence, highlighting the multistep nature of tumorigenesis[3,7,27,31,45]. The M-D-A sequence in Barrett's metaplasia may be a model for tumorigenesis in epithelial tissues in general. Most of the genes involved in the development of adenocarcinoma are normally responsible for the control of cell proliferation. Deviation from the strict control of proliferation leads to dysregulated division and, ultimately, cancer.

Cell signalling genes

These genes code for proteins that carry the mitogenic signals causing cells to proliferate. Inappropriate signalling can lead to continuous proliferation, a phenotype of cancer. *Fig. 1* shows a simplified cell signalling pathway, highlighting the role of some of the following genes.

c-erbB2 *gene*

The c-*erbB2* gene codes for a transmembrane receptor (including the *Her-2/neu* gene product) for external growth signals and is located at chromosome 17q21. Amplifications of this locus have been detected in oesophageal adenocarcinoma and appear to represent a late event in disease progression[46–48]. Overexpression of this gene has been correlated with poor survival[31,49].

Epidermal growth factor receptor gene

The epidermal growth factor receptor gene (*EGFR*; c-*erbB1*) is located at chromosome 7p12-13. Its amplification is detected in the majority of oesophageal adenocarcinomas[50,51] and represents a late event. Such amplification has also been correlated with the occurrence of lymph node metastasis[31].

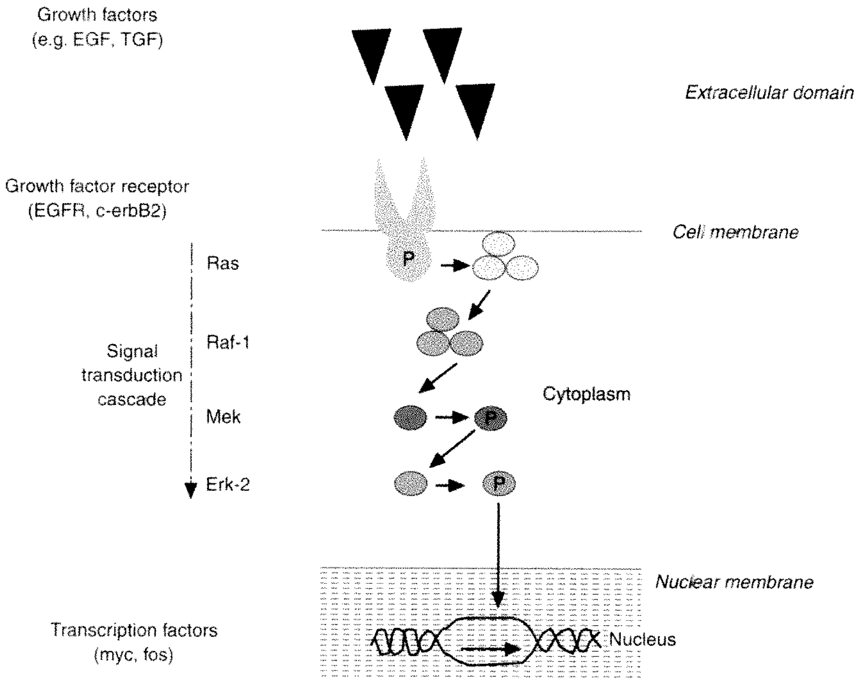

Fig. 1 Cell signalling pathway. In this example, extracellular growth factors stimulate cell proliferation by binding to and activating the appropriate growth factor receptor. This activation often involves autophosphorylation of tyrosine amino acids within the intracellular domain of the receptor. Phosphorylated tyrosine then acts as a signal for second messengers, which may involve pathways such as the cascade shown. In this pathway Ras passes on the growth signal to Raf-1 through its intrinsic guanosine 5′-triphosphatase activity. Raf-1 subsequently passes this signal (by serine kinase activity) on to mitogen-activated protein kinase (Mek) kinase. The Mek kinase has itself to be phosphorylated (P) before it can activate extracellular signal-regulated kinase (Erk) 2. Phosphorylated Erk-2 may subsequently activate the transcription factors c-fos and c-myc within the nucleus. EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; TGF, transforming growth factor

Transforming growth factor α *gene*

Transforming growth factor (TGF) α binds to EGFR and stimulates cell division[3]. Amplification of the TGF-α gene (at chromosome locus 2p13) has been detected in adenocarcinoma progression[52–54]. TGF-α is also implicated in the stimulation of vascular endothelial growth factor (VEGF) in Barrett's tissue[55]. VEGF has been correlated with vascularization and so may play a role in the angiogenesis of adenocarcinoma.

SRC

SRC is a tyrosine kinase involved in the epidermal growth factor signalling pathway and has been implicated in deregulating cell adhesion. The transcription factor c-myc can be activated by the SRC pathway, leading to cell proliferation[56]. SRC is overexpressed in metaplastic tissue[57,58], with a sixfold increase in kinase activity in adenocarcinomas[58]. Hence SRC activation may represent an early event in adenocarcinoma development.

K-ras *gene*

The K-*ras* gene encodes a protein involved in signal transduction. K-*ras* mutations have been detected in 40 per cent of HGDs and in 30 per cent of adenocarcinomas[59]. However, these mutations have not been detected in metaplastic tissue and are thought of as a late event in adenocarcinoma progression[60]. The K-*ras* gene has also been found to be amplified in 40 per cent of adenocarcinomas[61].

Cell cycle control genes

Cancer is often described as a disease of the cell cycle (*Fig. 2*). Cell cycle progression is controlled by cyclin-dependent kinases (CDKs) that promote progression, and CDK inhibitors that arrest progression. *Fig. 3* highlights the cell cycle control pathway involving the retinoblastoma (*Rb*) gene product. In progression of Barrett's metaplasia there appears to be an accumulation of cells in the S phase and the G2 phase[62], suggesting that cells are escaping the G1–G0 checkpoints.

Retinoblastoma gene

The *Rb* tumour suppressor gene, located on chromosome 13q14, mediates cell cycle progression (*Fig. 3*) and is also linked to apoptotic pathways mediated by the *p53* gene (via p14^{ARF}). The *Rb* gene locus shows loss of heterozygosity in 36–48 per cent of oesophageal adenocarcinomas[63–65] and is also detectable in metaplastic epithelium[27]. Loss of heterozygosity describes the loss of one of the two allelic copies of an autosomal gene. Abnormalities in the messenger RNA (mRNA) expression of *Rb* have also been detected in 28 per cent of adenocarcinomas[65,66].

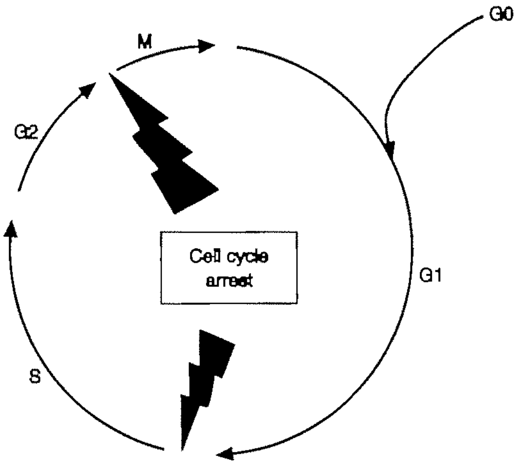

Fig. 2 The cell cycle, consisting of the gap 1 (G1) phase, the DNA synthesis (S) phase, the gap 2 (G2) phase and the mitosis (M) phase. Also shown are the two main checkpoints at G1–S and G2–M

Cyclin D1 and E genes

The cyclin D1 proto-oncogene lies on chromosome 11q13, a site often amplified in adenocarcinoma[67,68]. When cyclin D1 expression was examined in the early stages of Barrett's metaplasia progression, a significant increase was found, along with nuclear accumulation of the protein in 46 per cent of cases of metaplasia[69]. Cyclin D1 overexpression not only appears to be an early genetic change, but also significantly increases the risk of metaplasia progressing to adenocarcinoma[68]. A recent study showed that patients with overexpression of cyclin D1 were six to seven times more likely to develop adenocarcinoma than those without overexpression[70]. In addition, the cyclin E gene has been implicated in adenocarcinoma development and has recently been identified on chromosome 19q12, a site often amplified in adenocarcinoma[71].

p53 *gene*

See 'Genome maintenance/apoptosis genes' below.

p16 *gene*

The *p16* gene is a CDK inhibitor, located at chromosomal position 9p21. *p16* allelic loss has been demonstrated in 75 per cent of metaplastic tissues and precedes aneuploidy[72]. *p16* allelic losses in metaplastic and dysplastic Barrett's tissue are more prevalent than *p53* allelic losses, and are detectable throughout the metaplastic region[73]. Indeed, the loss of *p16* may represent the earliest genetic change in

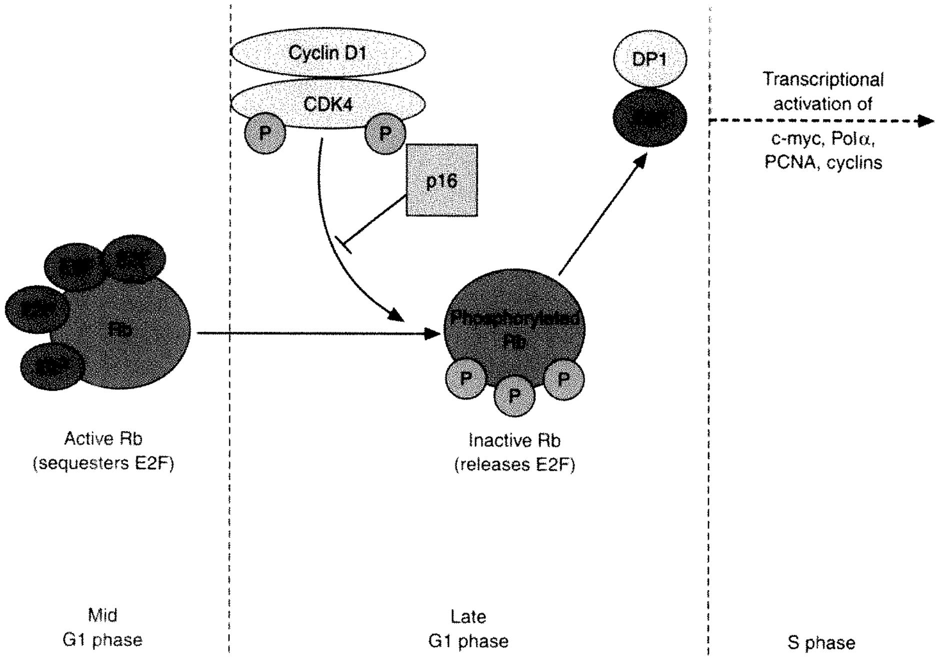
Cyclin D1
CDK4
P
P
p16
DP1
Transcriptional activation of
c-myc, Polα, PCNA, cyclins
Rb
Phosphorylated Rb
P
P
P
Active Rb (sequesters E2F)
Inactive Rb (releases E2F)
Mid G1 phase
Late G1 phase
S phase

Fig. 3 Control of cell cycle progression. In this example, entry into S phase of the cell cycle is controlled by the interaction of a number of gene products. The retinoblastoma protein (Rb) is a tumour suppressor that binds to the E2F transcription factor and prevents cell cycle progression. However, when the cell cycle approaches S phase, Rb is phosphorylated by cyclin-dependent kinase (CDK) 4, in conjunction with cyclin D1. Phosphorylated Rb releases E2F, which subsequently binds dimerization protein (DP) 1 and acts as a transcription factor initiating the transcription of key genes promoting S-phase transition. The CDK inhibitor p16 can block the phosphorylation of Rb and maintain cell cycle arrest; hence loss of the *p16* gene product can result in the upregulation of cell proliferation. Degradation of cyclin D by ubiquitin-mediated proteolysis negatively regulates the S-phase signal. Polα, DNA polymerase α; PCNA, proliferating cell nuclear antigen

progression of Barrett's metaplasia. Hypermethylation of the promoter region of the *p16* gene silences gene expression and so removes the gene product. In one study, nine of ten dysplastic tissues and eight of 21 metaplastic samples were shown to have aberrant *p16* promoter methylation[74,75].

p27 *gene*

The *p27* tumour suppressor gene, another CDK inhibitor, has been mapped to 12p13. In 83 per cent of adenocarcinomas immunohistochemical studies have shown that p27 protein levels are low, despite increased mRNA levels in all the cases[76]. For p27 to arrest the cell cycle, it must be localized to the nucleus, but 50 per cent of HGD cases have cytoplasmic localization of the protein, which renders it inactive[76]. This represents another mechanism of gene inactivation: inappropriate cellular localization of the gene product.

p21 *gene*

The *p21* gene is situated on chromosome 6p21 and encodes a *p53*-induced CDK inhibitor. Significant overexpression of this gene has been found at all stages of progression from LGD to adenocarcinoma[77,78], suggesting a *p53*-mediated block to cell cycle progression in response to DNA damage.

Cell adhesion genes

Cell adhesion is a mechanism that prevents invasion and metastasis by tumour cells. Hence cell adhesion genes have been implicated in the metastatic step of tumour progression. Decreased cell adhesion may also be important in the initial steps of progression of Barrett's metaplasia, allowing the drift of clonally derived cells along the epithelium to create a cellular field change.

Cadherin genes

Cadherins E and P are thought to be implicated in the metastasis of adenocarcinoma[79]. Loss of these proteins allows the migration of cells during metastasis[3]. There is progressive loss of cadherin expression throughout the M-D-A sequence[79–81]. Indeed, germline E-cadherin mutations may be responsible for the occurrence of adenocarcinoma in some patients[6].

Catenin genes

Catenin genes are implicated in the M-D-A progression via two mechanisms. First, catenins are involved in cell adhesion, acting in conjunction with cadherins. Second, catenin proteins interact with the adenomatous polyposis coli (APC) tumour suppressor protein, which mediates its own degradation. When APC function is lost, β-catenin accumulates and overflows into the nucleus, where it consequently acts as a transcription factor, driving the transcription of growth-promoting genes[3]. Increased catenin phosphorylation has also been shown throughout the M-D-A sequence[79,81], resulting in nuclear translocation[82] and transcription promotion. A recent report has suggested that β-catenin also activates transcription of cyclin D1, a proto-oncogene that drives cell cycle progression[83].

APC *gene*

The *APC* gene, mapped to 5q21, is involved in cell adhesion through its interaction with the catenin–cadherin proteins. In addition, APC protein binds to the ends of microtubules[84] that form the spindle apparatus during chromosome segregation, and so *APC* defects may mediate chromosomal instability during metaphase. The *APC* gene is a tumour suppressor gene and loss of *APC* alleles have been implicated in the M-D-A progression[85]: 5q is often lost in the progression to adenocarcinoma[27]. In one study, loss of heterozygosity of 5q was identified in HGD, adenocarcinoma and metaplastic tissue adjacent to HGD[86]. *APC* hypermethylation occurs at a high frequency in metaplasia and adenocarcinoma, and so appears to be an early event in neoplastic progression, causing loss of gene function[87,88].

DCC *gene*

The deleted in colonic cancer (*DCC*) gene encodes a cell adhesion molecule and is located on chromosome 18q21.1. Loss of heterozygosity of 18q21 increases progressively as metaplasia evolves to adenocarcinoma[89]. The fact that this has been detected in metaplasia as well as adenocarcinoma indicates that it may be an early change in the M-D-A sequence[34,90].

CD44

The CD44 receptor protein is involved in cell adhesion. The expression of CD44 variants increases progressively through the dysplasia sequence and their expression may be correlated with the aggressiveness of adenocarcinoma[91].

Genome maintenance/apoptosis genes

The DNA present in human cells is constantly subject to the effects of damaging agents. To maintain the correct copy number and sequence of key genes, there exist gene products that repair and maintain the genome. Without these proteins, errors in DNA replication are left unrepaired and accumulate in subsequent generations of cells.

p53 *gene*

The *p53* gene at chromosome 17p13 encodes a protein that monitors the integrity of the genome and halts cell cycle progression at G1 (via p21) if the genome is damaged, allowing time for DNA repair. If the genome cannot be repaired, *p53* promotes apoptosis. *p53* mutations may be present at all levels of the metaplastic segment, suggesting clonal expansion[27]. Allelic loss of 17p is also common, 79 per cent of adenocarcinomas having lost a *p53* allele[92]. *p53* mutations occur relatively early in the M-D-A sequence, being detectable in 40–88 per cent of adenocarcinomas, 30–66 per cent of HGDs[93–96] and up to 60 per cent of metaplasias[97]. Additionally, identical *p53* mutations have been found in adenocarcinomas and adjacent dysplastic tissue[92], suggesting a role for such mutations early in the progression to adenocarcinoma. In some studies, the same *p53* mutations found in dysplastic areas can be identified in metaplastic epithelia throughout the Barrett's segment[96]. Loss of *p53* function correlates with poor prognosis in Barrett's patients[98]. *p53* mutations in adenocarcinoma are clustered within so-called hotspots. These hotspots generally reflect the amino acid codons that best disrupt protein function. In *p53*, the hotspot codons are located in exons 5, 7 and 8 (codons 175, 248, 273)[85,97–101].

mdm2 *gene*

The *mdm2* gene, located at 12q15, plays an important role in the negative regulation of *p53*. *mdm2* has been shown to be overexpressed in adenocarcinomas[102], hence inactivating *p53*.

Telomerase

Telomerase enzymes maintain the ends of chromosomes, which naturally shorten during the cellular ageing process. Telomerase activity is thought to be involved in the progression to adenocarcinoma, as has been associated with cell immortality. Telomerase is normally expressed at low levels in gastrointestinal epithelial stem cells[103]. However, in one study 100 per cent of adenocarcinomas and HGDs were strongly positive for telomerase expression, and 70 per cent of metaplastic samples were moderately positive[23].

Bcl2 *gene*

The *Bcl2* gene at 18q21 inhibits apoptosis and is normally expressed in proliferative zones, such as the basal zone of the epithelium. In normal oesophageal epithelia, weak Bcl2 expression is present in the basal zone, but much stronger expression of the oncoprotein was found in 72 per cent of cases of metaplasia, 76–100 per cent of LGDs, 0–25 per cent of HGDs and 0–20 per cent of adenocarcinomas[104,105]. These data correlate with the observation that apoptotic cells are rarely seen during the M-D-A progression sequence[104]. Bcl2 overexpression can therefore account for reduced apoptosis in metaplasia and LGD.

Fas and Fas ligand

Fas ligand is produced by activated T cells and binds to Fas, a cell surface receptor, activating an apoptotic pathway. Fas ligand overexpression has been noted in metaplasia and is present more frequently in dysplasia[106]. In addition, the Fas membrane protein is also overexpressed in dysplasia[107]. A related receptor, decoy receptor 3, which sequesters Fas ligand, is also amplified in oesophageal tumours[108].

Miscellaneous genes

Inducible nitric oxide synthase gene

The inducible nitric oxide synthase (iNOS) enzyme[109] has been implicated as a contributory factor in the progression of metaplasia to adenocarcinoma. Nitric oxide is a potent reactive oxygen species, which is capable of inducing mutations in DNA[110]. When mRNA expression levels of iNOS were examined, 76 per cent of patients with metaplasia had increased levels, as did 80 per cent of those with adenocarcinoma[111].

Cyclo-oxygenase 2 gene

Enhanced cyclo-oxygenase (COX) 2 gene expression causes increased Bcl2 protein levels, inflammation, inhibition of immune surveillance and promotion of angiogenesis, thus driving tumorigenesis[112,113]. In the context of reflux-

Table 1 Chromosomal region abnormalities in progression of Barrett's metaplasia

Chromosome		Chromosomal region				
Gain	Loss	Gain	Loss	Method used	M-D-A stage	Reference
			9p21	LOH	Metaplastic epithelium	72
			5q, 13q, 18q	LOH	Metaplastic epithelium	27
	Y	8q23-24, 6p	18q12-22	CGH	Metaplastic epithelium	118
			5q21 17p11, 17p13, 18q21, 18q23	LOH	Metaplastic epithelium	34
	Y		5q, 9p, 17p	CGH	LGD	119
	Y	8q23-24, 2p23-24	9p13-pter, 17q	CGH	LGD	118
	Y	13q, 7p, 7q, 17q	18q, 7q, 5q, 9p, 17p, 12q	CGH	HGD	119
	Y	8q23-24, 20q, 2p23-24	5q13-23	CGH	HGD	118
			9p21, 17p13	LOH	HGD	73
	4, 18, Y	5q, 7q, 13q, 17q, X	3p, 7q, 9p	CGH	HGD	120
		4q, 21q	3p	CGH	HGD	118
7, 8, 11,17	Y			ISH	Adenocarcinoma	121
			4q (21-22, 32-33, 35)	LOH	Adenocarcinoma	122
	Y	8q23-24, 20q	4q	CGH	Adenocarcinoma	118
			17q24-25	LOH	Adenocarcinoma	121
			17p13, 17p13.1, 17p12, 17p11.2, 17q11.2	LOH	Adenocarcinoma	123
			17p, 5q, 9p, 13q, 18q	LOH	Adenocarcinoma	27
14, 20	4, 18, 21, Y	11p, 22p	1p22-33	Chromosome banding	Adenocarcinoma	124
			17p, 3p, 5q, 9p, 13q, 18q, 9q, 11p, 17q	Review of LOH data	Adenocarcinoma	3
12, 6, 7, 11				FISH	Adenocarcinoma	124
			17p, 13q, 5q, 18q	LOH	Adenocarcinoma	64
	Y	20q, 8q, 7p, 7q	18q, 9p, 4p, 4q, 16q	CGH	Adenocarcinoma	119
			9p, 17p, 5q	Microsatellite analysis	Cultured adenocarcinoma cell lines	101
	Y	8q23-24, 20q, 2p23-24, 6p, 10q, 17q11-22	4q, 5q13-23	CGH	Lymph node metastases	118
		3q, 7p, 17q, 22q	2q	CGH	Lymph node metastases	125

Earliest events are shown at the top, latest events at the bottom. M-D-A, metaplasia–dysplasia–adenocarcinoma; LOH, loss of heterozygosity; CGH, comparative genomic hybridization; (F)ISH, (fluorescence) *in situ* hybridization; LGD, low-grade dysplasia; HGD, high-grade dysplasia induced Barrett's metaplasia, *COX-2* expression is induced by bile acid *in vivo*[114] and *ex vivo*[115]. Additionally, the *COX-2* gene has been implicated in the M-D-A sequence because of its enhanced expression in metaplastic tissue[111,116]. COX-2 inhibitors may therefore represent promising tools in the clinical treatment of patients with Barrett's oesophagus[117].

Chromosomal regions implicated in Barrett's metaplasia

There are large inconsistencies in the literature relating to key chromosomal events driving the progression of Barrett's metaplasia to cancer. The causes of this confusion may stem from either the variety of different cytogenetic techniques used or interlaboratory variation[31]. In addition, flow-sorting the cells of Barrett's tissues produces an enriched subpopulation of tumour cells in which genetic alterations may be detected more readily[27]. This enrichment procedure is not performed in all cytogenetic studies and so may represent one source of variation. This review has aimed to overcome any variations in the literature by highlighting chromosomal changes that are consistently implicated in separate studies of the progression of Barrett's metaplasia (*Table 1*). One approach to determining the genetic steps involved in such progression is to identify the genetic alterations present in cultured Barrett's tumour cells. Cell lines derived from oesophageal adenocarcinomas have been karyotyped for common cytogenetic events. These studies detected aneuploidy in the cell lines as well as highlighting 9p (*p16* gene), 5q (*APC* and *MCC* genes) and 17p (*p53* gene) as important genetic alterations in disease progression[101].

Aneuploidy (abnormal chromosome numbers) has been strongly implicated in disease progression[126] and predicts adenocarcinoma development[27,127]. In turn, aneuploidy is preceded by the presence of increased tetraploid cells that can predict aneuploidy development[27]. These cells accumulate in the proliferating epithelium after the G1 checkpoint has been lost. Aneuploidy has been detected in adenocarcinoma, dysplastic tissue and metaplastic Barrett's epithelia[128], with some metaplastic epithelia containing several overlapping aneuploid cell populations[27,129].

The chromosomal regions most commonly lost in the early stages of the M-D-A progression are 5q (*APC*), 9p (*p16*), 13q (*Rb*), 17p (*p53*) and 18q (*DCC*)[27,34,72]. From *Table 1* it is possible to see that these early changes are also evident in dysplasia and adenocarcinoma, along with a host of late-stage rearrangements that may include 'passenger' alterations. Not all chromosomal loci implicated in disease progression can be linked to known cancer genes (e.g. 4q[92,130], 17p11[34,123], 18q23[34] and 17q[123,131]). Putative

Table 2 Location and function of key cancer-related genes

Chromosome	Location	Gene	Function
1	1p36	*E2F2*	Transcription factor
	1p13	N-*ras*	Oncogene
	1q13	*COX-2*	Inflammatory gene
	1q23	Fas ligand	Apoptotic signal protein
2	2p13	*TGFα*	Growth factor
	2p16	*MSH2*	Mismatch repair gene
	2p16	*MSH6*	Mismatch repair gene
	2q32	*PMS1*	Mismatch repair gene
3	3p25	*VHL*	Tumour suppressor gene
	3p22	β-Catenin	Cell adhesion gene
	3p21	*MLH1*	Mismatch repair gene
4	4q12	*PDGFR*	Platelet-derived growth factor receptor
	4p16	*FGFR*	Fibroblast growth factor receptor
	4q27	*MAD2*	Mitotic checkpoint gene
5	5p15	Telomerase	Telomere maintenance gene
	5q13	*MSH3*	Mismatch repair gene
	5q21	*APC*	Tumour suppressor gene
	5q21	*MCC*	G-protein gene
	5q31	α-Catenin	Cell adhesion gene
6	6p21	*p21*(*WAF*)	CDKN
	6q22	*E2F3*	Transcription factor
7	7p22	*PMS2*	Mismatch repair gene
	7p12	*EGFR*	Growth factor receptor
8	8q24	c-*myc*	Oncogene
9	9p21	*p15*	CDKN
	9p21	*p16*	CDKN
	9p22	*TGFβR*	Growth factor receptor
10	10q23	*PTEN*	Tumour suppressor gene
	10q24	Fas	Apoptotic signal receptor
11	11p15	*p57*	CDKN
	11q11	δ-Catenin	Cell adhesion gene
	11p15	H-*ras*	Oncogene
	11q13	Cyclin D1	Cell cycle gene
	11q22	*ATM*	DNA damage response gene
	11q22	Caspases 1,4,5	Apoptotic genes
12	12p13	*p27*	CDKN
	12p12	K-*ras*	Oncogene
	12q15	*mdm2*	P53 regulatory gene
	12q24	*INOS*	Inflammatory gene
13	13q13	*BRCA2*	Repair gene
	13q14	*Rb*	Cell cycle gene
16	16q22	E-cadherin	Cell adhesion gene
	16q22	*E2F4*	Transcription factor
17	17p11	*TCF2*	Transcription factor
	17p13	*p53*	Tumour suppressor gene
	17q2	*BRCA1*	Repair gene
	17q21	*c-erbB2*	Growth factor receptor
18	18q21	*Bcl2*	Antiapoptotic gene
	18q21	*Dpc4*	Growth factor effector
	18q21	*DCC*	Cell adhesion gene
19	19q12	Cyclin E	Cell cycle gene
	19q13	*TGFβ*	Growth factor
	19q13	*Bax*	Apoptosis gene
20	20q11	*E2F1*	Transcription factor
	20q13	*DcR3*	Fas ligand mimic

CDKN, cyclin dependent kinase inhibitor

genes on these chromosomal loci can be found in *Table 2*. As seen in *Table 1*, the studies performed on adenocarcinomas show a huge variety of chromosomes and chromosomal regions that are either amplified or deleted. As noted above, these probably represent some of the passenger changes arising in tumour cells as a consequence of reduced fidelity.

Chromosomal events linked with the metastatic stage include losses of 2q and gains of 3q, 7p, 17q and 22q, although these events were present against a background of genetic heterogeneity[125]. Finally, predisposition to Barrett's metaplasia, or more specifically to severe familial gastro-oesophageal reflux disease, has recently been mapped to chromosome 13q[132]. Hence this genetic location may contain a predisposing factor for patients progressing to metaplasia.

Discussion

Barrett's metaplasia represents an important premalignant condition affecting up to 3 per cent of the UK population. The fact that only 5–10 per cent of those with reflux acquire Barrett's metaplasia suggests the existence of host–environment factors which, if identified, may aid in the clinical management of reflux. Genetic studies identifying diagnostic and prognostic markers associated with the M-D-A sequence are in progress. These markers may subsequently be employed to identify the subset of patients at highest risk of developing adenocarcinoma. It is unlikely that a single genetic marker will be sufficient, as there appears to be no simple evolution of genetic alterations in disease progression; indeed, there appears to be a random accumulation of genetic changes in proliferating Barrett's epithelia[31,118], resulting in divergent clones that progress to tumour development. *Fig. 4* shows the current state of knowledge with respect to the key genetic changes implicated with each stage of disease progression; the complex nature of the changes may reflect the simultaneous disruption of several of the genetic pathways regulating cell proliferation. This genetic heterogeneity may ultimately be responsible for the poor responses of these tumours to therapy.

The early genetic events accompanying progression of Barrett's metaplasia are loss of the *p16* gene, loss of the *p53* gene, cyclin D1 overexpression and losses of the *APC*, *Rb* and *DCC* loci. Hence these genetic alterations may be suitable as markers of disease progression. Aneuploidy is another genetic alteration that is often noted at an early stage of disease progression and it may prove a useful marker of such progression. Indeed, the detection of

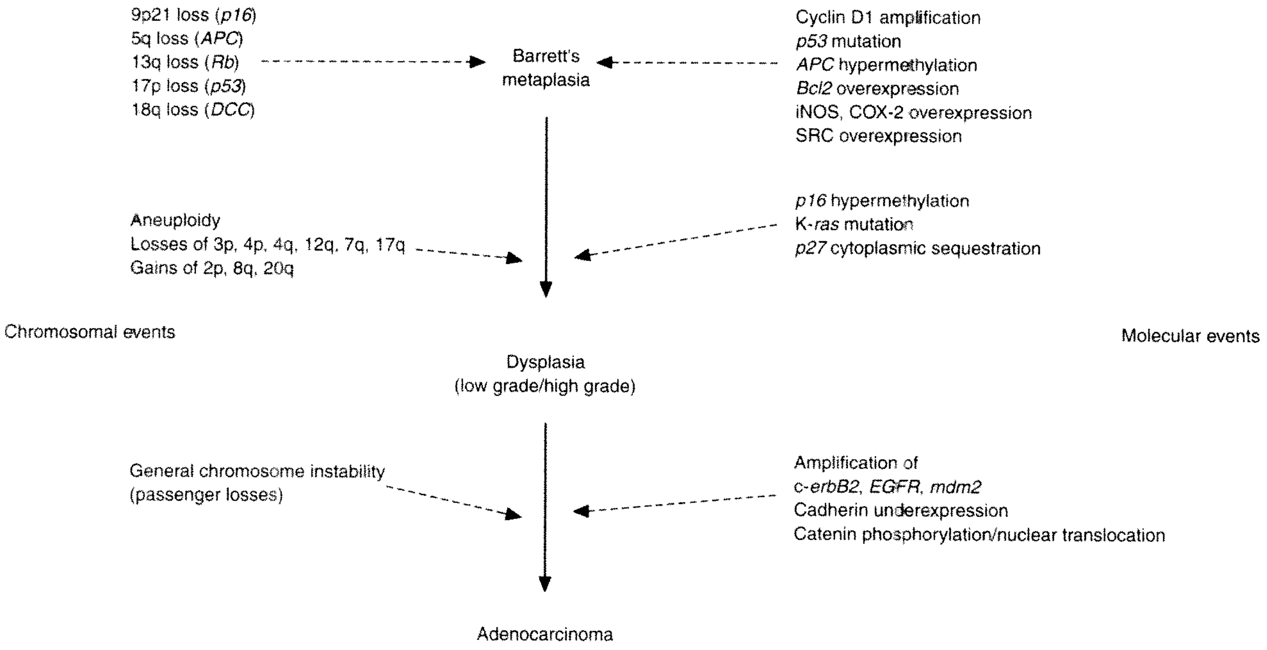

Fig. 4 Genetic events that contribute to the progression of Barrett's metaplasia to adenocarcinoma. The known molecular events (right side) and chromosomal events (left side) that accompany the histological stages of Barrett's progression are shown. *APC*, adenomatous polyposis coli gene; *Rb*, retinoblastoma gene; *DCC*, deleted in colonic cancer gene; *Bcl2*, B-cell leukaemia gene; iNOS, inducible nitric oxide synthase; *COX-2*, cyclo-oxygenase gene; SRC, tyrosine kinase of Rous sarcoma virus; c-*erbB2*, same as *Her-2* – human epidermal growth factor receptor; EGFR, epidermal growth factor receptor; *mdm2*, murine double minute 2

aneuploidy by flow cytometry has been advocated as a practical method for selecting high-risk patients for close surveillance[28]. It has been suggested that aneuploidy represents a driving force behind progression of Barrett's metaplasia[129], allowing the general genomic instability to occur that subsequently produces diverse cell subpopulations from which tumours select appropriate clones. In this model, the earliest, and perhaps the most important, genetic events are those that allow aneuploidy to arise (e.g. defects in chromosome segregation).

Many studies have identified genetic alterations in Barrett's oesophagus, but most have had very small sample sizes[31]. Larger studies are needed to validate postulated diagnostic markers. These large studies should also decrease interstudy variation, increase the statistical power of the analysis and prevent spurious findings biasing the collective data[4]. In the search for genetic markers of cancer progression, analysis of the earliest changes should be given the highest priority, as these changes will be free of 'passenger' alterations.

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Review

Homocysteine and occlusive arterial disease

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Background: An increased plasma level of homocysteine has been proposed as an independent risk factor for atherosclerosis; this review examines the evidence.
Methods: A Medline search was undertaken for English language articles on homocysteine and vascular disease. Further papers were identified by cross-referencing from the reference lists of relevant major articles.
Results: Although much interest has been generated about homocysteine and atherosclerotic disease, contradictory data exist regarding its role in disease progression. There is insufficient current evidence to regard increased homocysteine level as a causative factor in atherosclerotic disease.
Conclusion: It is not known whether lowering plasma homocysteine concentration will reduce cardiovascular risk in the long term. Until such data become available, there is no evidence for the widespread use of folic acid therapy to reduce cardiovascular disease risk.

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Introduction

A raised homocysteine concentration has been proposed as a risk factor for cardiovascular disease[1]. Such an increase is more prevalent in patients with coronary artery disease[2,3], cerebrovascular disease[4], peripheral vascular disease and thrombosis[5,6] than in normal subjects. Indeed, a raised homocysteine concentration is described as a strong predictor of death in patients with angiographically confirmed coronary artery disease[7], and those with symptomatic arterial occlusive disease of the lower limb and increased plasma levels of homocysteine seem to experience a more rapid progression of the disease[8]. A raised level of homocysteine has been estimated to be a greater risk factor (20–40-fold) than an increased level of cholesterol (1–3-fold), high blood pressure (8–18-fold) or cigarette smoking (3·5-fold)[1].

Despite this, conflicting recent evidence has emerged about the role of homocysteine in disease progression. One comparison of atherosclerotic risk factors found no significant difference in homocysteine levels between controls and diseased patients[9]. Initially an increased risk ratio for myocardial infarction was detected in relation to increased levels but, when confounding variables were accounted for, this was no longer significant[10]. It is not clear, therefore, whether there is sufficient evidence to regard an increase in plasma homocysteine concentration as a causative factor in atherosclerotic disease. It is also unclear whether dietary supplementation with folic acid would modify the disease process. This paper reviews the evidence for the clinical relevance of increased levels of homocysteine in the pathogenesis of atherosclerosis.

Biochemistry of homocysteine

Initially the association between homocysteine concentration and cardiovascular disease came from observations of children with homocystinuria[11], an autosomal recessive inborn error of metabolism resulting from a deficiency in one of three different enzymes. Homocystinuria results most commonly from a deficiency in cystathionine β-synthase (CβS). Individuals homozygous for the condition are unable to convert homocysteine to cystathionine, leading to high levels in the circulation and the resultant excretion of homocysteine in the urine. Homozygosity for methylenetetrahydrofolate reductase (MTHFR) and, rarely, methyltransferase deficiency also result in the clinical syndrome of homocystinuria, with skeletal and ocular defects and premature vascular events.

Under normal conditions, homocysteine concentration is maintained within a narrow range by the enzymes CβS and MTHFR, and is removed from the body by remethylation to methionine, or *trans*-sulphuration to products that are cleared in the urine. Remethylation occurs by the action of the enzyme MTHFR with folate as a co-substrate, and by methionine synthase with vitamin B_{12} as a co-factor. *Trans-*

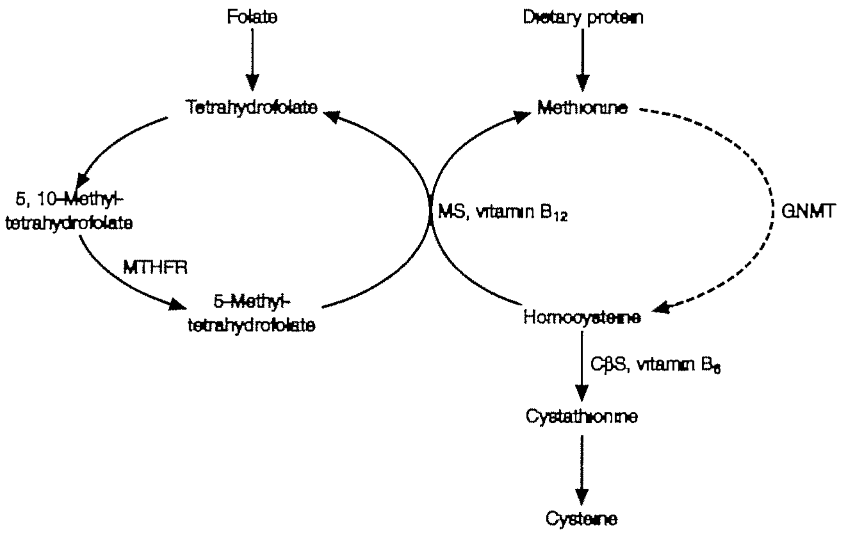

Fig. 1 Metabolism of homocysteine. CβS, cystathionine β-synthase; MS, methionine synthase; MTHFR, methyltetrahydrofolate reductase; GNMT, glycine *N*-methyltransferase

sulphuration requires CβS, which uses vitamin B_6 as a cofactor (*Fig. 1*).

Normal levels of fasting plasma homocysteine are considered to be between 5 and 15 μmol/l. Moderate, intermediate and severe increases in the concentration of homocysteine refer to levels of 16–30, 31–100 and more than 100 μmol/l respectively[12]. Moderate increases in homocysteine level may result from a combination of genetic and dietary factors. A thermolabile variant of MTHFR with decreased basal activity has recently been found in both normal subjects (5 per cent) and patients with vascular disease (17 per cent)[13]. The common thermolabile MTHFR variant results from a cytidine to thymidine point mutation at nucleotide 667, which significantly reduces the basal activity of the enzyme[14]. There has been much debate in the recent literature with regard to the significance of the common C667T mutation of MTHFR and its role in mild hyperhomocysteinaemia[15–18]. A meta-analysis by Brattstrom[19] of 14 studies of this specific mutation demonstrated no difference in frequency of expression of the mutant allele between controls and patients. Importantly, no increase in cardiovascular risk could be identified, but the power of this study was low.

High homocysteine levels can occur as a result of dietary vitamin deficiency without an inborn error of metabolism. Dietary deficiencies in vitamin B_{12} and folic acid produce milder increases in plasma homocysteine concentration than that found in homocystinuria, and dietary intake and plasma levels of these vitamins are inversely related to levels of plasma homocysteine. Homocysteine levels begin to rise at plasma levels of folate and vitamin B_{12} (and vitamin B_6) that are regarded as within the low normal range[20,21]. Folic acid and vitamin B_{12} protect against the build-up of homocysteine in the blood by conversion to methionine. Vitamin B_6 also prevents the accumulation of homocysteine by converting it to cysteine and other compounds excreted in urine.

Experimental evidence for the role of homocysteine in vascular disease

Evidence suggests that an increase in homocysteine concentration may induce atherosclerosis by a combination of endothelial injury[22], smooth muscle proliferation[23–25], platelet activation and thrombogenesis[5,26,27]. Endothelial dysfunction may arise from a disturbance in the oxidative metabolism of homocysteine[28]. Its oxidation stimulates the production of reactive oxygen species, such as superoxide anion, hydrogen peroxide and hydroxyl radical, that exert potent oxidative stress[29,30].

At physiological concentrations, homocysteine interacts with nitric oxide to produce *S*-nitrosohomocysteine, which amplifies the vasoprotective effects of nitric oxide. Stamler *et al.*[31] observed that normal endothelial cells detoxify homocysteine by releasing nitric oxide, which in turn leads to the formation of *S*-nitrosohomocysteine. This chain of reactions decreases the production of hydrogen peroxide following oxidation of sulphydryl groups and represents a protective mechanism against the adverse effects of homocysteine, but prolonged exposure and high homocysteine concentrations result in impaired nitric oxide production. Thus, chronically raised homocysteine levels appear to result in a self-perpetuating cycle that progressively overwhelms the capacity of the endothelial cells to reduce the toxicity of homocysteine[31]. The results of these *in vitro* studies imply that homocysteine at high concentration may produce many of the pathological processes associated with atherosclerosis. The homocysteine concentrations used in many of these experiments, however, were in the pharmacological range and not relevant to the levels encountered in clinical practice. The results of these studies must, therefore, be interpreted with caution.

Endothelial damage may have an adverse effect on flow-mediated dilatation of large arteries, an endothelium-dependent mechanism believed to be an early indicator of the atherosclerotic process. Woo *et al.*[22] demonstrated an impairment of brachial artery endothelium-dependent dilatation in patients with raised homocysteine levels (mean(s.d.) 34·8(8·5) μmol/l). Although there were only 14 participants in the study, endothelial dysfunction was demonstrated *in vivo* at clinically relevant concentrations.

Vascular smooth muscle proliferation with subsequent formation of extracellular matrix collagen is an integral component of atherosclerosis. Homocysteine increases DNA synthesis with subsequent smooth muscle proliferation[23,24] in a dose-dependent manner at plasma concentrations ranging from 25 to 500 μmol/l[25].

The vascular endothelium maintains a non-thrombogenic surface by the antithrombin III and thrombomodulin–protein C anticoagulant pathways. Following treatment with homocysteine, endothelial cells have been shown to exhibit enhanced factor V activity (a procoagulant factor)[26] and reduced production of protein C[27]. The concentrations used (up to 1 mmol/l) were again dissimilar to those found in the clinical setting, but even moderate increases in plasma homocysteine level have been shown to be a risk factor for deep vein thrombosis[5].

Leucocyte adhesion to endothelium is an important part of the inflammatory process of atherogenesis. The accumulation of leucocytes in inflamed tissues results from adhesive interactions with endothelial cells in the microcirculation[32]. Pruefer *et al.*[33] demonstrated increased adhesion and trans-endothelial migration of leucocytes when the mesentery of rats was perfused with plasma concentrations of homocysteine ranging from 1 to 5 mmol/l. Immunohistochemical staining demonstrated significantly increased P-selectin and intercellular adhesion molecule (ICAM) 1 expression on the intestinal venules after homocysteine perfusion[33]. This finding was not, however, supported by work from Dudman and Hale[34], who found no increase in ICAM-1 expression on the endothelium following treatment with homocysteine. The possibility that homocysteine acts indirectly by activating leucocytes, which in turn interact with and induce pathological changes in the vascular endothelium, was then investigated, and endothelial–leucocyte interaction was seen to be mediated by the neutrophil-docking protein complex CD11b–CD18[35].

Notwithstanding the impressive list of atherosclerotic effects of homocysteine, its biological relevance remains questionable. The effects described above occur only at concentrations of homocysteine in the pharmacological range. It is unclear how lower concentrations might be associated with an increased risk of atherosclerotic disease. Neutrophils, when treated with clinically relevant concentrations of homocysteine, demonstrate no upregulation of adhesion marker expression[36]. This questions the role of neutrophils in homocysteine-mediated injury.

Clinical evidence for the role of homocysteine in vascular disease

Although considerable *in vitro* evidence exists to support a link between raised homocysteine concentration and atherosclerosis, clinical studies have produced mixed results. Several prospective analyses of homocysteine and cardiovascular disease have been reported[7,37,38]. Verhoef *et al.*[39] observed virtually no association between plasma homocysteine level and risk of angina pectoris with subsequent coronary artery bypass surgery. Folsom *et al.*[40] found that fasting total homocysteine concentration was associated positively and relatively strongly at high levels with the age-, race- and centre-adjusted incidence of coronary heart disease in women, but not in men. However, adjustment for other risk factors abolished the association, suggesting that total homocysteine concentration was not independently associated with coronary heart disease. In the Physicians' Health Study, with a 5-year follow-up, plasma homocysteine levels above the 95th percentile of the control distribution were associated with a 3·4-fold increased risk of myocardial infarction[3]; however, the relative risk was 1·7 with 7·5 years of follow-up and this was no longer statistically significant. Failure to show an association may be explained by a variation in homocysteine levels within subjects over time, which may have attenuated the effect. The results, however, question the role of homocysteine in atherosclerotic disease. Perhaps homocysteine is not causative, but merely a marker for cardiovascular disease.

Graded associations are described between plasma homocysteine concentration and carotid artery intimal–medial wall thickening[41] and the prevalence of carotid artery stenosis[42]. In a sample of patients drawn from the Physicians' Health Study, Verhoef and co-workers[43] studied 109 patients who developed an ischaemic stroke over a 5-year period. They observed no association between increased plasma homocysteine level and the risk of ischaemic stroke. A prospective study of homocysteine and atherosclerotic disease in middle-aged men and women in Finland showed no association with myocardial infarction or stroke[44]. Highlighting the conflicting evidence, a strong independent association between homocysteine level and stroke was observed in a sample of middle-aged British men, albeit in a retrospective study, by Perry *et al.*[45], consistent with the hypothesis of a causal link between raised homocysteine concentration and stroke. After adjustment for confounding variables, Taylor *et al.*[46] reported an increase of 1 µmol/l in the plasma homocysteine concentration to be associated with a 5·6 per cent increased likelihood of death from vascular disease. In their retrospective study, although a similar age of onset of symptoms was noted in patients with raised and normal homocysteine levels, a significant progression in lower-limb occlusive disease and coronary artery disease was found in those with raised levels. No such association was noted for cerebrovascular disease[8]. This work was followed by a prospective study of 351 patients with symptomatic peripheral arterial disease. No statistically significant relationship between decreasing ankle : brachial pressure index and increasing carotid stenosis was found in patients with increased homocysteine levels, despite a mean follow-up of 37 months[46].

Diet and hyperhomocysteinaemia

As noted above, genetic factors alone do not always account for raised levels of homocysteine. Other conditions that may be associated with high levels are advanced age[47], hypothyroidism[48], impaired renal function[49], systemic lupus erythematosus[50] and certain drugs, such as theophylline[51], methotrexate[52] and L-dopa[53]. The relationship between diet and homocysteine levels has been well documented[54,55]. Folic acid and vitamin B_{12} levels, through their involvement with the remethylation of homocysteine to methionine, relate inversely to homocysteine concentration in plasma. Evidence is accumulating that low levels of folate in the blood are associated with cardiovascular disease[56,57]. A study of elderly Framingham Heart Study participants showed that deficiencies of folate, vitamin B_6 and vitamin B_{12} intake, and in their plasma levels, were common in that population and were associated with increased plasma homocysteine levels[58]. These increases could be treated successfully, at least in the short term, with vitamin supplementation[59]. The use of a combination of all three vitamins did not appear to reduce the homocysteine level to a significantly greater degree than folic acid alone[60–62]. Although the addition of vitamin B_{12} to the diet may not significantly reduce the homocysteine level, 1 mg per day of oral vitamin B_{12} in addition to folic acid may avoid the theoretical risk of neuropathy due to unopposed folic acid therapy in patients deficient in vitamin B_{12}, even in those with intrinsic factor deficiency or malabsorption states[63].

The most effective means of improving vitamin intake was investigated by Riddell *et al.*[63]. Their data provided strong evidence that folic acid fortification of breakfast cereals and the use of folic acid supplements were the most effective means of reducing total homocysteine concentration. The US Food and Drug Administration guidelines on folic acid fortification of grain in 1996, although issued to reduce the risk of neural tube defects, also served to improve folate status and reduce the incidence of increased homocysteine concentration. The fortification consists of adding 140 µg folate per 100 g grain and, following its introduction, in those who did not use vitamin supplements the prevalence of low folate concentration decreased by 92 per cent, and the prevalence of high homocysteine concentration decreased by 48 per cent[64]. Work by Rimm *et al.*[65] supported these findings, but found that the policy of grain fortification at this level would increase the average intake by only 100 µg folate per day. The lowest risk of coronary heart disease among women is associated with an intake of folate above 400 µg/day.

Evidence that lowering homocysteine concentration with vitamin supplementation favourably affects the evolution of atherosclerosis is limited. In an observational study of 38 patients with plasma homocysteine levels above 14 µmol/l, high doses of folic acid (2·5 and 5 mg/day) together with vitamins B_6 and B_{12} resulted in a reduced rate of progression of carotid plaque (determined by ultrasonography) compared with pretreatment values, after a mean follow-up of 4 years[66]. There are no data, however, to suggest that a similar effect on other peripheral arterial diseases will occur with folic acid supplementation. Owing to the lack of prospective evidence that folate supplementation will modulate the atherosclerotic process, estimation by Boushey *et al.*[67] that the addition of folic acid to the food supply will prevent as many as 50 000 coronary artery-related deaths annually is questionable.

The role of folate in lowering cardiovascular risk is not limited to reducing plasma homocysteine level. Folic acid supplementation has been shown to improve endothelial function in patients with hypercholesterolaemia[68] and in cigarette smokers[69], independent of its effect on homocysteine concentration. This improvement in endothelial function is likely to result from a variety of mechanisms. Folic acid may increase nitric oxide formation by stimulating the recycling of tetrahydrobiopterin, an essential cofactor of endothelial nitric oxide synthase[70]. Alternatively, folates may reduce the degradation of nitric oxide by reducing superoxide production by nitric oxide synthase[71].

Increased homocysteine concentration may be a marker of poor diet, a known risk factor in atherosclerosis. As noted above, diets deficient in folate are frequently associated with raised homocysteine concentration[58]. Emerging evidence also suggests an important role for dietary nutrients other than folic acid in modulating the atherosclerotic process, in particular the n-3 fatty acids and antioxidant vitamins[72].

Conclusion

Although a raised plasma level of homocysteine has been postulated as an independent risk factor for atherosclerotic disease, evidence from experimental and clinical studies is contradictory. Moreover, despite the possibility of reducing homocysteine concentration by an increased intake of folic acid, it is not known whether a reduction of plasma homocysteine concentration by diet or vitamin therapy will reduce cardiovascular risk in the long term. It must also be considered that any benefit from folic acid supplementation may stem from the direct protectant effect of folate on the vasculature, independent of any homocysteine-lowering effect. Regardless of its mechanism of action, folate therapy warrants further study.

Selected patients with a personal or family history of premature arterial disease, and those with renal failure or who are taking certain drugs, may be wise to increase their intake of vitamins through the consumption of fortified foods or vitamin supplements. However, such treatment remains experimental pending the results of prospective trials on homocysteine reduction and the evolution of arterial occlusive disease. Until these data become available, there is little evidence for the widespread use of folic acid in an attempt to reduce the risk of atherosclerotic disease.

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The colorectal adenoma–carcinoma sequence

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Background: It is widely accepted that the adenoma–carcinoma sequence represents the process by which most, if not all, colorectal cancers arise. The evidence supporting this hypothesis has increased rapidly in recent years and the purpose of this article is to review this evidence critically and highlight its clinical significance.

Methods: Medline searches were used to identify recent key articles relating to the adenoma–carcinoma sequence. Further pertinent articles were obtained by manual scanning of the reference lists of identified papers.

Results: The evidence supporting the adenoma–carcinoma sequence can be classified as epidemiological, clinicopathological and genetic. The most recent and largest body of data relates to molecular genetic events and their cellular effects; however, many other approaches, such as cytogenetics, molecular cytogenetics and cytometry, have also yielded valuable information.

Conclusion: Recent work continues to support the adenoma–carcinoma sequence, but there is a paucity of data on the interrelationship between different genetic mutations and on the relationship between molecular and other types of genetic abnormalities. The clinical utility of the observations described has yet to be fully realized and global genetic analysis of colorectal tumours may prove to be central in rational adenoma management.

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Introduction

Colorectal cancer is the second leading cause of cancer-related death in the Western world; in the UK there are currently around 30 000 new cases per annum and 17 000 related deaths[1]. In recent years our understanding of the cellular and molecular events underlying the development of colorectal cancer has improved immeasurably but, despite this and advances in surgery, radiotherapy and chemotherapy, the average 5-year survival rate remains around 40 per cent[1].

One of the most important fundamental concepts in colorectal cancer to emerge in recent years has been the adenoma–carcinoma sequence, a term that describes the stepwise progression from normal to dysplastic epithelium to carcinoma associated with the accumulation of multiple clonally selected genetic alterations. This concept not only provides an excellent model to study the genesis of invasive cancer, but also affords a means of preventing colorectal cancer by endoscopic removal of precursor lesions.

The appropriate management of individuals with precursor adenomatous polyps (adenomas) is of the utmost importance. It is known that after removal of such polyps 30–35 per cent of patients will have further adenomas detected at 3–4 years[2–4] and this has led to a policy of endoscopic surveillance for all adenoma-bearers[5]. However, given that approximately 40 per cent of the Western population will develop adenomas[6–8] and only 3 per cent will go on to suffer from colorectal cancer, it is clear that only a small proportion of adenomas progress to malignancy. Unfortunately, there are no reliable criteria available that can predict adenoma progression or recurrence, and the important questions of which individuals require follow-up, how they should be followed and for how long remain largely unanswered.

Although the adenoma–carcinoma sequence has not been proven directly, there is considerable indirect evidence to support it from a range of epidemiological, clinical, histopathological and genetic studies. The purpose of this article is to review the current knowledge relating to the sequence in order to address potential ways forward in the management of colorectal neoplasia, with particular emphasis on possible novel genetic markers of disease progression.

Epidemiological and clinicopathogical evidence

Age distribution curves for adenomas and carcinomas show that the prevalence of both increases with increasing age, but adenomas are recognized and their prevalence peaks at least 5 years earlier than that of colorectal cancers[9]. Furthermore, the varying prevalence of adenomas in different geographical regions correlates with the colorectal cancer incidence in those regions[10]. Studies performed before the era of colonoscopic polypectomy examined the progression of polyps left *in situ* over variable periods of time; both polyp growth and regression, and colorectal carcinoma arising at the site of the index polyp, were observed[11–14].

Histopathological studies have demonstrated foci of malignancy within colorectal adenomas in 0·2–8·3 per cent of cases[15–18]. Conversely, remnants of adenomatous tissue contiguous with invasive cancer have been identified in 14–23 per cent of all colorectal cancers[9,19]. Furthermore, the presence of benign tumour adjacent to cancer has been shown to correlate inversely with the extent of spread of the malignant tumour. Adenomatous remnants have been found in 57–60 per cent of cancers limited to the submucosal layer, compared with only 7–17 per cent of cancers with extramural spread[19,20], suggesting that as cancers invade through the bowel wall they also expand on the mucosal surface, replacing existing benign tumour. In surgical resection specimens and during endoscopic examinations adenomas are found to coexist with cancer in approximately 30 per cent of cases[21–23], and patients who have colorectal cancer and simultaneous adenomas have been shown to be at increased risk of synchronous and metachronous cancer compared to those without coexisting adenomas[22]. Similarly, adenomas are encountered more frequently in patients with synchronous primary cancers than in those with a single primary cancer[23].

In clinical studies, the anatomical distribution of adenomas and cancers is similar, both occurring more frequently distal to the splenic flexure[10,16,18,22,24]; adenomas of the left colon more often contain severe dysplasia or invasive adenocarcinoma[16,18,24]. Finally, from a practical viewpoint, endoscopic removal of adenomatous polyps appears to reduce the long-term risk of colorectal cancer[25–29]. Of particular importance are the 18-year follow-up results of a large randomized controlled trial, which show that annual or biennial faecal occult blood testing significantly reduces the incidence of colorectal cancer[30]. The most plausible explanation of these findings is that identification and removal of adenomatous polyps following large bowel investigations led to the reduction in colorectal cancer incidence.

Genetic evidence

The concept of cancer arising from 'genetic abnormalities' has existed for many years. The first actual demonstration of such an abnormality was the discovery of the Philadelphia chromosome in chronic myeloid leukaemia in 1960[31]. This small karyotypic marker originates from a translocation between chromosome 22 and chromosome 9, i.e. t(9;22)(q34;q11)[32]. Cytogenetic recognition of this abnormality has since led to the identification of the underlying molecular pathology, a fusion gene that brings the *abl* oncogene under the regulatory influence of the oncogene *bcr*. The Philadelphia chromosome exemplifies how analysis of the genetic changes in a tumour cell can be performed at several different levels. In colorectal cancer, genetic changes have been studied most commonly at the gene or primary DNA structure level, using molecular techniques, but investigations have also been carried out at the cytogenetic level by microscopic examination of metaphase chromosomes, and at the cellular level by cytological or flow cytometric assessment of total nuclear DNA content.

Another important approach for identifying cancer-related genetic changes is the recognition of genes that are differentially expressed in normal and tumour tissue. With the development of complementary DNA (cDNA) microarrays this is now feasible and has recently been applied to colorectal cancer.

Molecular genetic evidence

The genes of interest that are involved in genetic alterations may be classified into three types: oncogenes, tumour suppressor genes and DNA repair genes[33]. Under normal conditions, oncogenes stimulate appropriate cell growth, but mutation or overexpression of oncogenes results in 'gain of function' and causes cells to continue to grow in the absence of growth signals. Tumour suppressor genes normally inhibit progress through the cell cycle or promote programmed cell death (apoptosis), but when their expression is absent, as a result of mutation or actual allelic loss, there is loss of normal inhibitory control. Finally, DNA repair genes are involved in controlling the rate of mutation of other genes. When mutated, repair genes are unable to repair errors and so mutations in oncogenes and tumour suppressor genes accumulate at an accelerated rate.

An alternative class of indirectly acting cancer susceptibility genes has been proposed by the 'landscaper' hypothesis[34]. In this model, based on studies of juvenile polyposis syndrome (JPS), it is suggested that the primary oncogenic effect of the mutation is on stromal cells, resulting in an altered stromal environment that increases the cancer susceptibility in overlying epithelial cells. Recent work on JPS[35] has, however, questioned this hypothesis[36].

In 1990 Fearon and Vogelstein[37] proposed a genetic model for colorectal tumorigenesis. This model postulated that mutational activation of oncogenes, coupled with mutational inactivation of tumour suppressor genes, leads to the development of colorectal tumours. Although these alterations often occur in a sequence that parallels the clinical progression of the tumour[37,38], it was the total accumulation of changes rather than their order that was suggested to be important. The key oncogene in this model was *ras*, and the key tumour suppressor genes were proposed to reside on chromosomes 5q, 17p and 18q. Other somatic alterations, such as loss of DNA methyl groups and overexpression of certain proteins, were also thought to be involved. In the past decade a multitude of studies has generated extensive data regarding the function and interactions of these key genes, providing support for, and clarification of, this model. However, evidence is now emerging that an alternative pathway exists in a subset of colorectal tumours that less frequently involves the aforementioned genes and often involves mismatch repair genes[39]. Current molecular genetic knowledge relating to the adenoma–carcinoma sequence will be reviewed in the following sections, with an emphasis on the frequency with which each alteration occurs at different stages of the adenoma–carcinoma sequence.

APC

One mutation known to occur early in the adenoma–carcinoma sequence affects the adenomatous polyposis coli (APC) tumour suppressor gene located on chromosome 5q21[40,41]. Germline mutations of this gene are responsible for familial adenomatous polyposis (FAP), an autosomal dominant disorder characterized by the development of hundreds to thousands of colorectal adenomas appearing in adolescence or early adulthood. If untreated, FAP inevitably leads to colorectal cancer, usually by the third or fourth decade of life[42]. The product of the *APC* gene is a large 312-kDa protein composed of 2843 amino acids. It is a multifunctional protein with several domains that is known to interact with a number of other proteins, including β-catenin, glycogen synthase kinase (GSK) 3β, end binding protein (EB) 1, human homologue of the *Drosophila* discs large tumour suppressor protein (hDLG) and Bub kinases[43–46]. One function important to colorectal tumorigenesis is the ability of *APC* to regulate intracellular β-catenin levels[47]. Non-mutated (wild-type) APC protein forms a complex with β-catenin and GSK-3β[43], which leads to an enhanced rate of β-catenin degradation[47] by the ubiquitin–proteasome pathway[48]. Mutated APC proteins lack this ability and intracellular β-catenin accumulates[47]. The importance of β-catenin relates to its ability to bind to the T-cell factor (TCF) family of transcription factors and activate gene transcription[49]. Increased β-catenin–TCF-mediated transcription has been demonstrated in tumours with *APC* mutations and in tumours with β-catenin mutations[50,51]. Recently, c-*myc*, a gene known to have powerful oncogenic functions[52,53], has been identified as a target gene of this increased transcriptional activity[54].

Another function of the APC protein that has been largely neglected but may be important in colorectal tumorigenesis is its association with microtubules and its role in chromosome segregation. Wild-type but not mutant forms of APC protein have been shown to bind to, and promote the assembly of, microtubules[55,56]. Another protein, EB-1, originally identified by its physical association with the C-terminal of APC protein[45], is also known to localize to cytoplasmic and spindle microtubules; it has been suggested that EB-1 may play a role in connecting APC to microtubules[57]. Furthermore, two recent studies have demonstrated that APC, during mitosis, accumulates at the ends of microtubules embedded in kinetochores, and that mutant cells lacking the microtubule-binding domain of APC display defective chromosomal segregation and chromosomal instability[44,58]. This evidence suggests that wild-type APC protein plays a role in stabilizing the ends of kinetochore microtubules and assists in their attachment to chromosomes. Finally, when localized to kinetochore microtubules, APC complexes with the mitotic checkpoint proteins Bub-1 and Bub-3[44]. Although the function of this interaction remains undetermined, it is interesting that mutational inactivation of Bub-1 has been associated with chromosomal instability in colorectal cancer cell lines[59].

APC mutations or allelic losses of 5q are observed in 40–80 per cent of colorectal cancers[38,60–62] and are found at a similar frequency in adenomas[38,60,62,63]. Interestingly, although *APC* mutations occur at a similar frequency at all stages of colorectal tumour progression, allelic loss or loss of heterozygosity (LOH) has been shown to increase in frequency from early adenomas through to invasive carcinomas[62]. Furthermore, mutated *APC* has been demonstrated in adenomas as small as 0·5 cm[60], reinforcing the belief that such mutations are involved early in the adenoma–carcinoma sequence. Finally, about half of those tumours with only wild-type *APC* have been found to harbour β-catenin mutations[51,64], suggesting that these mutations can substitute for *APC* mutations in colorectal carcinogenesis. However, it is intriguing that β-catenin mutations occur significantly more frequently in small adenomas (12·5 per cent) than in large adenomas (2·4 per cent) or invasive cancers (1·4 per cent)[65]. This observation suggests that adenomas with β-catenin mutations may be less likely to progress to invasive cancer.

K-ras

A further genetic alteration believed to occur early in the adenoma–carcinoma sequence is an activating mutation of the oncogene K-*ras*. This oncogene, one of three of the *ras* gene family, encodes a 21-kDa protein (ras p21) involved in signal transduction of regulatory pathways critical for normal proliferation and differentiation[66,67]. It is a guanosine 5′-triphosphate (GTP)-binding protein, located at the cytoplasmic aspect of the cell membrane, with intrinsic GTPase activity that is regulated by several other proteins[68]. When bound to GTP the ras protein is active, but becomes inactive when GTP is hydrolysed to guanosine 5′-diphosphate[69]. All known carcinogenic mutations of the K-*ras* oncogene affect codons in the GTP-binding domain, decrease its GTPase activity, and result in a constitutively active ras protein[68].

Activating K-*ras* mutations occur in 35–42 per cent of colorectal carcinomas[38,70–73] and are observed at a similar frequency in large adenomas[38,74,75]. The relatively high frequency of *ras* mutated adenomas supports the hypothesis of this oncogene as an early participant in the adenoma–carcinoma sequence. However, as K-*ras* mutation is less common in small adenomas[38,63,74,75], it seems that while this alteration confers a growth advantage it is unlikely to be an initiating factor in colorectal tumorigenesis.

Jen and colleagues[63] analysed small hyperplastic and adenomatous polyps from cancer-bearing colon for *APC* and K-*ras* mutations. *APC* mutations were found to be closely associated with dysplasia, as 82 per cent of adenomas and no hyperplastic polyps had detectable mutations. Conversely, K-*ras* mutations were almost equally common in non-dysplastic and dysplastic polyps (22 and 25 per cent respectively). Furthermore, all K-*ras* mutated dysplastic polyps had concomitant *APC* mutations, suggesting that mutation of K-*ras* alone is insufficient for the onset of dysplasia.

Mutations of K-*ras* have been investigated as potential predictors of metachronous adenomas[76]: K-*ras* mutations were significantly associated with larger metachronous adenomas but did not provide a significant additional contribution to the prognostic value of size and villous component in predicting metachronous adenomas. Interestingly, Morris *et al.*[77] have shown that K-*ras* mutation occurs significantly more often in adenomas from colons with a synchronous cancer than in comparable adenomas from cancer-free individuals. Furthermore, K-*ras* mutations have been identified in histologically normal mucosa[78,79] from cancer-bearing colons, and it has been suggested that mutant K-*ras* may be a useful biomarker to identify persons at higher risk of, or for early detection of, colorectal cancer[78,79].

p53

The *p53* gene is the gene most frequently altered in human cancers[80]. Located on the short arm of chromosome 17, *p53* was initially implicated in colorectal cancer as a result of the frequent loss of 17p in allelic loss and cytogenetic studies[38,81–84]. The biochemical function of p53 as a sequence-specific DNA-binding protein and transcription factor controlling the expression of a large number of genes has been discussed extensively in recent reviews[85,86]. In brief, p53 has been labelled the ‘guardian of the genome’ because of its ability to block cell proliferation in the presence of DNA damage, to stimulate DNA repair and to promote apoptotic cell death if repair is insufficient[87]. Loss of p53 function therefore contributes to propagation of damaged DNA to daughter cells and, consistent with this, mutation of *p53* has been shown to precede aneuploid clonal divergence[88]. However, the relationship between p53 and aneuploidy is not a simple one, as crude aneuploidy has been observed in the absence of *p53* overexpression[89], chromosomal aberrations have been demonstrated in colorectal cancers with only wild-type *p53*[90,91] and *p53* is known to be mutant in some cell lines that are chromosomally stable[92]. Such disparities may be partly explained by different mutations having different downstream effects in the p53 pathway, thereby resulting in different tumour phenotypes.

Functional inactivation of p53 most often occurs as a result of missense mutations in the DNA-binding domain, but it can also result from oncogenic viral or cellular protein interaction[93,94]. In normal cells p53 protein has a very short half-life but, as mutant p53 protein is resistant to mdm-2–ubiquitin-mediated proteolysis, it accumulates within the cell[95]. This feature has been exploited clinically to identify *p53* mutations indirectly by immunohistochemical detection of the protein[96]. Many reports on the frequency of *p53* mutations in colorectal tumours have been based on immunohistochemical evidence of overexpression, some have employed direct DNA sequencing and others have investigated 17p allelic loss. Thus it can be said that ‘alteration’ in *p53* or 17p allelic loss has been reported in 4–26 per cent of adenomas[38,74,75,97–101], in approximately 50 per cent of invasive foci within adenomatous polyps[99,100], and in 50–75 per cent of adenocarcinomas[38,72,98,101–103]. This distribution of results has led to the belief that functional inactivation of p53 protein is associated with the transition from adenoma to carcinoma.

18q loss

While 17p is the most common region of allelic loss in colorectal cancer, the second most common region is chromosome 18q, which is lost in approximately 70 per cent of cases[38,103]. 18q loss is observed in 10–30 per cent of 'early' adenomas and the incidence rises to approximately 60 per cent in 'late' adenomas[38,103]. Originally the candidate tumour suppressor gene in this region was thought to be the 'deleted in colorectal cancer' (*DCC*) gene[104]. Research has since shown this gene to encode for a component of a receptor complex that mediates the effects of netrin-1, a molecule involved in axon guidance[105]. Experiments involving the mouse homologue of *DCC* have failed to support a tumour suppressor function for *DCC*[106] and mutant alleles of *DCC* are rarely demonstrated in colorectal tumours showing 18q allelic loss[107].

Meanwhile, other tumour suppressor genes in this region of interest have been identified, including *SMAD2* and *SMAD4*. The latter gene was originally identified due to its frequent loss in pancreatic cancer and was previously designated *DPC4*[108]. The protein products of both genes are intracellular mediators of the inhibitory transforming growth factor (TGF) β signalling pathway, which exerts a wide range of effects on many cells, including regulation of cell growth, differentiation, matrix production and apoptosis[109,110]. Targeted deletion of *SMAD4* in human colorectal cancer cell lines abrogates TGF-β signalling[111], and mutant SMAD2 and SMAD4 proteins are known to be targets for rapid degradation by the ubiquitin–proteasome pathway[112]. Mutations of *SMAD2* and *SMAD4* have been identified in several human cancers[113], including colorectal carcinomas and cell lines[113–116]. There appear to be no data on the prevalence of *SMAD* gene mutations in sporadic adenomas, but it is interesting that germline mutations of *SMAD4* have recently been observed in around one-third of individuals with the rare familial disorder JPS[117]. Finally, mutations of TGF-β type II receptor itself have been demonstrated in 90 per cent of colorectal cancers showing microsatellite instability[118], providing further support for the role of this pathway in colorectal carcinogenesis. Mutations of this gene are discussed further in the section on microsatellite instability.

Methylation status

A potential alternative mode of altering genes during tumorigenesis is an epigenetic process known as DNA methylation. This mechanism is known to play a role in genomic imprinting and normal embryonic development[119,120], and it has been implicated in differential gene expression[121]. However, its role in tumorigenesis remains undefined[122,123]. Compared with DNA from adjacent normal tissue, DNA from benign and malignant colonic tumours displays global hypomethylation[124,125]. Accordingly, the agent 5-azacytidine, which inactivates DNA methyltransferase, the enzyme responsible for methylation, has been shown to be carcinogenic[126]. Conversely, constitutive overexpression of mouse DNA methyltransferase causes neoplastic transformation[127], and reduction of DNA methyltransferase activity (genetically and pharmacologically) inhibits adenoma formation in mice with one mutant *APC* allele[128]. Furthermore, moderately increased levels of human DNA methyltransferase have been detected in normal mucosa from tumour-bearing colons, and markedly increased levels have been detected in adenomas and carcinomas[129].

In contrast to widespread changes in methylation, alternative lines of investigation have focused on hypermethylation of specific sequences of DNA. Hypermethylation of the promoter region of several tumour suppressor genes has been described and shown to be associated with transcriptional silencing[130–136]. Of particular interest is the *APC* gene, the promoter region of which is hypermethylated in 18 per cent of sporadic carcinomas and in 18 per cent of sporadic adenomas; in all cases there was associated loss of *APC* expression[136]. This evidence suggests that hypermethylation is an important mechanism for altering *APC* function and that it occurs as an early event in colorectal tumorigenesis. Finally, hypermethylation has increasingly been associated with sporadic colorectal cancer showing microsatellite instability (MSI)[131–133,137] (see below). Of specific importance is the *bMLH1* gene, which is hypermethylated in the majority of sporadic microsatellite unstable colorectal cancers (cell lines and primary tumours), a finding that is often associated with loss of *bMLH1* expression[131–133].

Microsatellite instability

Recently, an alternative pathway of tumorigenesis for a subset of colorectal tumours has been proposed, characterized by the presence of MSI. Microsatellites are a type of DNA that consists of tandem repeats, usually between one and five base pairs, repeated many times[138]. Hundreds of thousands of microsatellites are found interspersed throughout the human genome and are particularly prone to errors during DNA replication. Such errors are usually repaired by mismatch repair (MMR) proteins but, in the absence of competent MMR function, microsatellite errors accumulate[138]. When these errors are sufficiently frequent, the term MSI or replication error positive (RER$^+$) is applied. More recently, tumours showing MSI have been further classified into those exhibiting low and high levels of instability (MSI-L and MSI-H respectively)[139]. When a cell is MMR deficient it is not only microsatellites that are at risk of replication error but all nucleotide repeat sequences, including those in coding regions of key regulatory genes. Thus MSI can be interpreted as a marker for a state of hypermutability or a ‘mutator phenotype’[140,141].

MSI is observed in almost all adenocarcinomas from patients with hereditary non-polyposis colorectal cancer (HNPCC)[142–144] and occurs in 10–15 per cent of sporadic colorectal cancers[39,142–148]. HNPCC is an autosomal dominantly inherited condition that accounts for approximately 5 per cent of all cases of colorectal cancer[138]. It is characterized by relatively small numbers of adenomas, the development of colorectal cancer at an early age, a predominance of tumours in the proximal colon and is sometimes associated with extracolonic tumours, such as gastric and endometrial cancer[138,149]. Interestingly, while MSI has been described relatively frequently in sporadic gastric and endometrial cancers, it is less common in other solid cancers[150,151]. The presence of MSI-H in sporadic colorectal cancer and HNPCC correlates significantly with a number of clinical and pathological features, including proximal location[39,142,145,148,152–155], diploid DNA content[39,148,152,155,156], a more favourable Dukes' stage[152], an improved survival rate[145,148], a poor or mucinous differentiation[148,154,155,157], and the presence of a Crohn's-like inflammatory infiltrate[155,157]. Studies of MSI in sporadic adenomas have produced variable results, with instability ranging from 0 to 25 per cent[143,144,158–161]. Such variability may be explained partly by the different techniques used, different thresholds used to define instability, and the location of the studied polyps. A recent study has considered these issues and reported MSI in only 1·8 per cent of all adenomas and in 2·5 per cent of proximal adenomas[153]. In patients with HNPCC, however, MSI has been reported in more than 50 per cent of adenomas[143,162]. These observations suggest that MSI in this proposed pathway occurs early in familial cases but is a relatively late event in sporadic cases.

To date, mutations in five human MMR genes have been described: *hMSH2*, *hMLH1*, *hPMS1*, *hPMS2* and *MSH6*[138]. The majority of those with HNPCC have a germline mutation in one of these genes, with more than 90 per cent of cases involving *hMSH2* or *hMLH1*[163]. Furthermore, many HNPCC tumours harbour mutations in both alleles, one germline and one somatic, or total absence of the wild-type allele, indicating that both copies of an MMR gene are inactivated before a tumour develops[164,165]. Somatic mutations of these genes are found less frequently in sporadic MSI cancers[147,166], suggesting that novel genes may be involved. However, lack of *hMLH1* or *hMSH2* expression has been demonstrated in 95 per cent of sporadic MSI-H tumours[152], and loss of *hMLH1* expression is known to be associated with hypermethylation of the promoter region of this gene (see above).

Of particular interest is the high frequency of TGF-β type II receptor (RII) mutations in MSI-positive colorectal tumours. The gene coding for this receptor contains mononucleotide repeats, analogous to microsatellites, and so is particularly sensitive to defects in DNA mismatch repair. Inactivating mutations of RII have been found in 90 per cent of colorectal cancers showing MSI; in most cases both alleles are affected[118]. RII mutations have also been studied in MSI-positive adenomas at various stages of progression[161]. The earliest stage at which RII mutations were detectable was in high-grade dysplastic adenomas, and in adenomas containing a focus of invasive carcinoma the prevalence of RII mutations was approximately 75 per cent. These observations suggest that mutation of TGF-β RII is a critical step in MMR-deficient colorectal tumour formation, that RII behaves like a tumour suppressor and that its mutation correlates strongly with the progression of adenoma to carcinoma. However, it is interesting that RII mutations are also common in MSI-positive gastric cancer but rare in MSI-positive endometrial cancer[167]. These findings suggest that RII mutations do not simply reflect MSI but are instead selected for, and contribute to, the genesis of gastrointestinal cancers.

Cytogenetic evidence

It is now generally believed that most human cancers are genetically unstable. It has been argued that such instability, resulting from an increased mutation rate early in tumorigenesis, is the defect underlying tumorigenesis[168]. Instability is thought to occur at two distinct levels, the nucleotide level and the chromosomal level[169]. In colorectal tumorigenesis, nucleotide instability seen as MSI affects only a minority of tumours, but is well characterized. In contrast, chromosomal instability is poorly understood but is likely to affect most colorectal tumours. Alteration in chromosome number (aneuploidy) is known to occur in most colorectal tumours and, indeed, in virtually all human solid cancers[169]. However, the molecular basis for these observations remains unknown and is probably multifactorial. Interestingly, a defect in chromosomal segregation, resulting in gains and losses of chromosomes, has been demonstrated in colorectal cell lines without MSI, and this chromosomal instability persisted throughout the lifetime of the tumour[170].

Cytogenetic investigation (karyotyping) of tumours entails direct microscopic analysis of structural and numerical chromosomal abnormalities. Tumour cells from direct or cultured tissue are placed on glass slides and stained to produce a banding pattern on the condensed chromosomes that enables their identification. Structural changes, such as deletions, translocations, inversions and duplications, can then be analysed by microscopic inspection. Cytogenetic analysis provides diagnostic and prognostic information for a wide variety of human diseases and malignancies, but it is time-consuming and highly specialized work. Furthermore, tissue culture of solid tumours is notoriously difficult. Despite these problems a large number of colorectal tumours have been successfully karyotyped and characteristic patterns of chromosomal abnormalities have been reported[171].

Over 90 per cent of all colorectal cancers show chromosomal aberrations; only a minority have a normal karyotype[172,173]. The most common numerical aberrations, as summarized by Bardi and colleagues[174], are loss of chromosome 18 (26 per cent of tumours) and gain of chromosome 7 (25 per cent). Other common losses include chromosomes Y (15 per cent), 17 (13 per cent), 14 (12 per cent) and 22 (12 per cent), and other commonly gained chromosomes are 20 (14 per cent) and 13 (12 per cent)[174]. Although less frequent, many structural rearrangements have been reported that generally result in loss of 1p and gain of 1q, loss of 8p and gain of 8q, loss of 13p and gain of 13q, and loss of 17p and gain of 17q[174].

Chromosomal anomalies occur in 44–80 per cent of all sporadic colorectal adenomas, and numerical aberrations correlate with a villous component and with the degree of dysplasia[175–177]. The most frequent abnormality in adenomas, trisomy 7, is found in 40 per cent of lesions and is often the only aberration[171]. Trisomy 7 as a sole anomaly occurs in a wide variety of tumour types and has also been reported in non-neoplastic diseases, such as Dupytren's contracture and atherosclerotic plaques; its tumorigenic importance remains undetermined[178]. Interestingly, a candidate colorectal tumour suppressor gene, 'downregulated in adenoma' (*DRA*), which is expressed exclusively in colonic mucosa, has been localized to chromosome 7[179,180], and downregulation of this gene correlates with tumour progression[181]. As in colorectal cancer, chromosomes 13 and 20 are often gained in adenomas (30 and 17 per cent respectively) and chromosome 18 is frequently lost (13 per cent)[174]. Conversely, chromosome 14 is gained in 13 per cent of adenomas but is more frequently lost in carcinomas[174]. The single most common structural abnormality in adenomas is deletion of 1p, and multiple studies have shown that band 1p36 is always deleted, suggesting that a candidate tumour suppressor gene resides in this region[182].

Molecular cytogenetic evidence

Although karyotyping has provided vital information about the key chromosomal changes underlying colorectal cancer, it is associated with inevitable selection bias in favour of relatively simple karyotypes, as more complex karyotypes cannot be analysed with conventional techniques. In recent years there has been a rapid expansion of new fluorescence *in situ* hybridization techniques that combine standard cytogenetics with molecular genetics. These techniques, termed 'molecular cytogenetics', have greatly facilitated the genetic analysis of solid cancers. In particular, comparative genomic hybridization (CGH), a relatively new molecular cytogenetic technique, has overcome many of the drawbacks of conventional cytogenetic analysis of solid tumours, as it avoids the need for tissue culture and does not require analysis of complex karyotypes[183]. In a single experiment, CGH screens the entire genome for losses and gains of genetic material in a tumour, and provides a map of the chromosomal regions involved. However, it cannot detect rearrangements, such as inversions and balanced translocations. Briefly, tumour DNA is labelled with a green fluorochrome and normal DNA with a red fluorochrome. Equal quantities of each are then hybridized to normal metaphase chromosomes on a glass slide. The green : red fluorochrome ratio is analysed digitally and reflects the gain or loss of genetic material in corresponding regions of tumour DNA.

Since its introduction almost a decade ago[183], CGH has been performed on a wide variety of human tumours[184]. For colorectal tumours CGH data exist for low- and high-grade adenomas, carcinomas, liver and lymph node metastases, and for carcinomas showing MSI. Reid *et al.*[89] found that the number of genetic aberrations increased with progression from low-grade adenoma to high-grade adenoma to carcinoma. Only three of 14 low-grade and five of 12 high-grade adenomas showed chromosomal abnormalities by CGH, compared with 14 of 16 carcinomas. The most frequently gained chromosomal regions were 20q, 13q, 8q, 7p and 7q, and frequent losses were observed at 8p, 18q, 4q and 17p. Furthermore, the frequency of specific alterations increased with advancing stages of tumour progression; for example, gain of chromosome arm 7p occurred in 7 per cent of low-grade adenomas, 33 per cent of high-grade adenomas and 50 per cent of carcinomas. Similarly, Meijer *et al.*[185] found that the average number of chromosomal aberrations increased from adenoma to carcinoma; frequent gains involved 13q, 7p, 7q, 8q and 20q and losses most often occurred at 18q, 4q and 8p. In this study, however, at least one chromosomal abnormality was detected in every adenoma. The abnormalities detected by CGH in these studies are consistent with results of karyotypic characterizations of colorectal adenomas and carcinomas[174]. In their study of 45 primary colorectal carcinomas by CGH, De Angelis *et al.*[90] found that both DNA aneuploid tumours and tumours with *p53* mutations were significantly associated with an increased number of chromosomal aberrations.

CGH studies comparing primary colorectal carcinomas and their associated metastases have produced varying results. Korn and colleagues[186] found no consistent difference between primary tumours and synchronous liver metastases, whereas Al-Mulla *et al.*[187] found, in all cases studied, that liver or lymph node metastases had acquired new genetic alterations not present in the primary lesion. In addition, this latter group showed that loss of 17p was significantly associated with Dukes' stage C and lymph node metastases, while gain of chromosomal arms 6p and 17q was associated with Dukes' stage 'D' and liver metastases. However, Paredes-Zalgul *et al.*[188] showed that the number of losses per tumour was significantly higher for liver metastases and identified genetic alterations that were unique to metastatic lesions, including loss of 17q, 11q and 9q.

Finally, chromosomal differences between colorectal carcinomas with and without MSI have been demonstrated using CGH[189]. None of the MSI-positive tumours was found to have gained chromosomal material and only two of six had a single chromosomal segment deletion; 11 of 12 MSI-negative tumours had gains and losses. This observation is consistent with the correlation between MSI-positive tumours and a diploid DNA content[142,148,152,155,156], suggesting that this group of colorectal cancers does not exhibit gross chromosomal instability.

Cytometric evidence

DNA cytometry, an established technique for quantifying aneuploidy, measures total nuclear DNA content, typically using a flow cytometer. The number of cells in each category of DNA content is displayed in a histogram. The technique has the advantage of ease of use, and it can be applied retrospectively to archival paraffin-embedded material. Like cytogenetics, it provides a global assessment of abnormality, but only of numerical changes; it also has the disadvantage of poor sensitivity. DNA cytometry, while easy to perform and informative in its own right, has been used largely to complement the other approaches. Aneuploidy measured by this method is present in 50–75 per cent of colorectal carcinomas, is rarely found in Dukes' A carcinoma but is found in 55 per cent of Dukes' B and in 83 per cent of Dukes' C carcinomas[190]. Some studies have shown aneuploidy in colorectal cancer to be significantly associated with poor prognosis[191,192], but others have failed to support this finding[193,194].

The frequency of aneuploidy in adenomas is reported to be much lower[195,196] and correlates best with size (0 per cent in lesions of less than 1 cm, 16–52 per cent in lesions greater than 2 cm) and to a lesser degree with histological type (2 per cent in tubular adenomas, 11 per cent in villous adenomas)[196]. No increase in aneuploidy in adenomas associated with synchronous carcinoma has been described[196]. Owing to the inherent limitations of this technique, some tumours with subtle chromosomal aberrations or tumours with large chromosomal rearrangements that do not lead to changes in total DNA content, such as balanced translocations, will go undetected and be classified as diploid.

Gene expression evidence

In recent years significant advances have been made in the development of techniques to detect differential gene expression between cell populations. Among these new techniques are the cDNA and oligonucleotide microarrays[197], which allow the expression levels of multiple genes to be monitored in parallel. A cDNA microarray consists of thousands of DNA samples attached to a small glass slide. RNA from two samples is labelled with green or red fluorescent dyes; the samples are mixed and allowed to hybridize to the microarray. The ratio of red : green fluorescence at each site reflects the relative expression of that gene in the two samples. The second type of microarray is composed of oligonucleotides synthesized on to a silica slide, and fluorescently labelled cDNA derived from the test sample is hybridized to the array.

Microarrays have not yet been applied extensively to human colorectal tumours. However, a number of reports[198–201], analyses of cell lines[202–205] and studies of other tumour types[206] have shown its potential. Of particular interest is work by Notterman *et al.*[201], who examined 18 colon adenocarcinomas, four adenomas and paired normal tissue for each tumour by oligonucleotide array. They found 19 transcripts (0·5 per cent of those detected) whose expression was higher and 47 transcripts whose expression was lower in carcinomas compared with corresponding normal tissue. Several of the abnormalities detected in carcinomas were also present in adenomas, for example overexpression of the metalloproteinase matrilysin. This suggests that dysregulation of those specific genes may play a role at an early stage of colorectal tumorigenesis. It seems likely that microarray technology will play an increasingly important role in the coming years and new information management technology will facilitate interpretation of the data generated.

Conclusion

Over the past decade our understanding of colorectal tumorigenesis has advanced rapidly; the knowledge gained has strengthened the concept of the adenoma–carcinoma sequence, and has reinforced the practice of polypectomy and postpolypectomy surveillance. Unfortunately, however, this knowledge remains incomplete and so management of colorectal tumours remains suboptimal. In particular, it is widely recognized that only a small proportion of adenomas progresses to invasive cancer but, as yet, there is no reliable means of identifying this subgroup of patients. In addition, the related problem of identifying patients with adenomas who are likely to develop further tumours after polypectomy remains unsolved. Although there is growing optimism that tumour profiling may be of value in this respect, the clinical potential of this approach has yet to be realized (probably as individual aberrations have been studied in isolation). In the authors' view, the best chance of progress in adenoma management is to select a panel of genetic abnormalities that, based on current knowledge, may be associated with adenoma recurrence or progression. Using this panel to study large cohorts of patients with adenoma prospectively may enable markers of high risk to be identified. It is essential that such research continues in parallel with careful histopathological studies.

In recent years the molecular approach to colorectal tumorigenesis has been favoured, and research on the model proposed by Fearon and Vogelstein[37] has led to many important discoveries on the structure, function and interactions of certain key genes. The frequencies with which these genes are mutated at different stages of the adenoma–carcinoma sequence are consistent with, and serve to strengthen, the original model (*Table 1*). Furthermore, many novel genes have been described and an alternative pathway based on the presence of MSI has been proposed. However, despite the existence of a large body of knowledge on the various genetic abnormalities that have been identified in adenomas and carcinomas, there is a surprising lack of data relating to the coexistence of these abnormalities in individual tumours. To correct this, multiple analyses need to be performed on a comprehensive range of tumours; only then will patterns of genetic abnormalities emerge that might inform clinical practice.

Table 1 Frequency of common genetic aberrations in sporadic human colorectal tumours

	Frequency (%)	
Mutation or aberration	Adenoma	Adenocarcinoma
APC mutation	59–82[60,62,63]	52–60[60–62]
5q allelic loss	27–29[38,62]	36–56[60–62]
β-Catenin	2–12[64,65]	1–17[64,65]
K-*ras*	12–34 (< 1 cm)[38,75] 44–59 (> 1 cm)[38,75]	35–41[38,70–73]
p53 mutation or overexpression	4–26[74,75,97–101]	51–74[72,98,101,102]
17p allelic loss	14[38]	71–75[38,103]
18q allelic loss	11–70[38,103]	73[38,103]

It should also be stressed that the molecular analysis of tumours is essentially reductionist because mutations of oncogenes and tumour suppressor genes are often evaluated in isolation, without acknowledgement of numerous other coexistent genetic abnormalities. Cytogenetic techniques are complementary to molecular techniques as, by the detection of genome-wide numerical and structural chromosomal changes, they can identify previously unsuspected abnormalities. However, cytogenetic techniques clearly underestimate the occurrence of genetic abnormalities, such as small deletions or point mutations. Furthermore, each of these approaches will inform, and be informed by, the advent of new techniques such as expression microarrays. Thus, in order to obtain a balanced picture of the genetic mechanisms underlying cancer it is imperative that investigations continue at molecular, chromosomal and cellular levels; each should facilitate interpretation of the others and, ultimately, the knowledge gained may be accurately and safely utilized in the clinical setting.

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Late results concerning feasibility and compliance from a randomized trial of ultrasonographic screening for abdominal aortic aneurysm

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Background: The study was an update at 10 years of a randomized trial of the efficacy of screening for abdominal aortic aneurysm (AAA). The extent of benefit, feasibility and compliance were examined, and reasons why this intervention may fail a proportion of those screened were identified.

Methods: A total of 6058 men aged 65 years and over were randomized to a group invited to attend ultrasonographic screening or to a control group. The mortality rate from AAA in the two arms of the trial was compared using a Poisson model. Analyses were by intention to treat.

Results: There was a 21 per cent reduction in mortality rate from AAA over the 10-year follow-up (relative risk 0·79 (95 per cent confidence interval 0·53 to 1·40)). The observed relative mortality reduction peaked at 4 years with a 52 per cent reduction in the study group. Eighteen of 24 AAA deaths in the study group were among those who did not attend the first screen, or failed to comply with the follow-up protocol.

Conclusion: A greater awareness of the benefits of full participation in a screening programme could provide a larger and sustained mortality reduction.

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Introduction

The Chichester study, a randomized trial of the efficacy of screening for abdominal aortic aneurysm (AAA) by ultrasonography, began in 1988 and published 5-year results in 1995[1]. Men and women aged 64 years and over in the Chichester region were randomized to an invitation to ultrasonographic screening or to a control group. AAA mortality data in the two groups were compared to estimate the possible benefits of such a screening strategy.

With more extensive mortality data now available via the Office of National Statistics (ONS) flagging system, the present analysis of the Chichester data set now includes 10 years of follow-up data. The questions of feasibility, compliance and extent of benefit were examined, and reasons why this intervention fails a proportion of those screened were identified.

Patients and methods

The detailed methodology and primary results of the Chichester study were published in 1995[1]. Both men and women aged 65 years and over were included, but in the present analysis only men were considered as the incidence of AAA in women was too small to favour screening.

A total of 6341 men were recruited; 6058 were eligible for inclusion and were randomized to a group invited to attend screening by ultrasonographic imaging, or to a control group. Three thousand men were randomized, stratified by year-of-birth cohorts from general practitioner (GP) lists, to the screening arm, of whom 2212 (74 per cent) accepted the invitation. The control group contained 3058 men who were not contacted in any way, but all deaths were flagged by the ONS.

An AAA was defined as an aortic diameter greater than or equal to 30 mm. Men were referred to a vascular surgeon for consideration of elective AAA repair if the aortic diameter

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The Editors have satisfied themselves that all authors have contributed significantly to this publication

exceeded 60 mm, if the aneurysm grew by more than 10 mm per year (or pro rata over smaller intervals), if symptoms developed or if an iliac aneurysm (defined as 30 mm or more in diameter) was found. Otherwise, men identified with an aortic diameter measuring between 30 and 44 mm were recalled annually for repeat ultrasonographic screening, while those with an aorta measuring between 45 and 59 mm were invited at 3-month intervals.

The date of randomization was defined *post hoc* as the first ultrasound scan date in each practice. Thirty-five men in the screening group and 28 in the control group died before the date of randomization and were excluded from analyses.

The mortality rate in the two groups was compared using a Poisson model with the number of deaths, detected through the ONS flagging system, as the numerator and the number of person-years of follow-up as the denominator. The primary outcome measure was death from AAA (International Classification of Diseases 9, 441·3–6); analysis was by intention to treat.

Results

A summary of the 10-year results of the trial is shown in *Fig. 1*.

Death from abdominal aortic aneurysm

From the beginning of the study to 31 March 1999 there were 31 deaths from AAA in the control group and 24 in the study group, representing a 21 per cent reduction in mortality rate over the whole 10-year follow-up (relative risk 0·79 (95 per cent confidence interval 0·5 to 1·4)). Cumulative mortality by year since randomization is shown in *Fig. 2*. Mortality in the study group took 4 years to separate from that in the control group, with a maximum

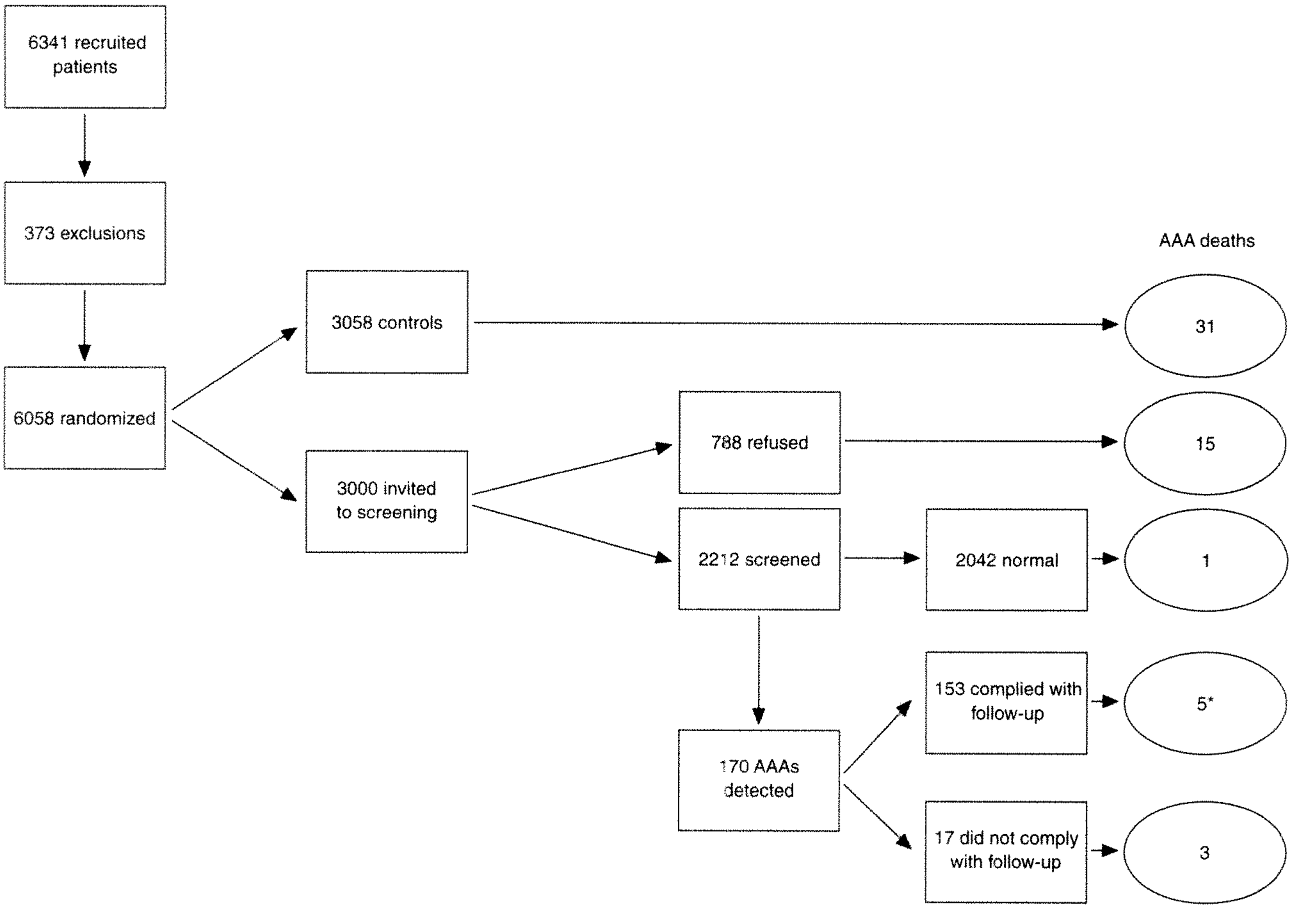

Fig. 1 Flow diagram of the randomized trial of ultrasonographic screening for abdominal aortic aneurysm (AAA). *Three unfit; two did not reach the criteria for elective surgery

reduction in mortality rate of 52 per cent observed 4 years after randomization, representing seven deaths in the study group compared with 15 in the control group.

Of the 24 patients who died from AAA in the study group over the 10-year interval, 15 died having declined the initial invitation for screening and three having refused screening recalls or surgery. Of the remaining six patients who died from AAA in the study group, three were deemed unfit for surgery, one man who had a normal aortic diameter at the initial screen died 3·5 years later from an AAA, and two had not reached the size criteria for elective surgery.

In the control group, 24 men died from ruptured AAA without reaching the operating theatre and seven died within 30 days of emergency surgery

Surgery for abdominal aortic aneurysm

Of the 82 known AAA operations in the study, 33 were carried out in the control group and 49 in the study group (*Table 1*). Seventeen of 33 operations in the control group were elective compared with 36 of 49 in the study group. In the study and control groups combined, there were no deaths among the 53 men who underwent elective AAA surgery. Focusing on the 49 operations in the screening arm, 39 were in men who complied with the protocol and reached criteria for consideration for elective surgery. Four men had emergency repair of an AAA that had not reached the criteria for elective surgery, five had refused the initial scan and one man had an AAA that had not been visualized at the initial scan (*Table 1*).

Considerations for elective surgery in the screening group

Of the 170 men found to have an AAA, 82 reached one or more of the criteria for elective surgery. Eighteen men met the size criterion for elective surgery at the initial scan and a further 64 went on to reach criteria for elective surgery during follow-up. Subsequent decisions and outcomes for those considered for elective surgery are shown in *Table 2*.

Discussion

The Chichester study was not powered to detect a moderate effect size (more than a 20 per cent reduction in mortality rate in the study group) and so the results, based on the relatively small number of participants recruited to the trial, did not reach statistical significance. They provided,

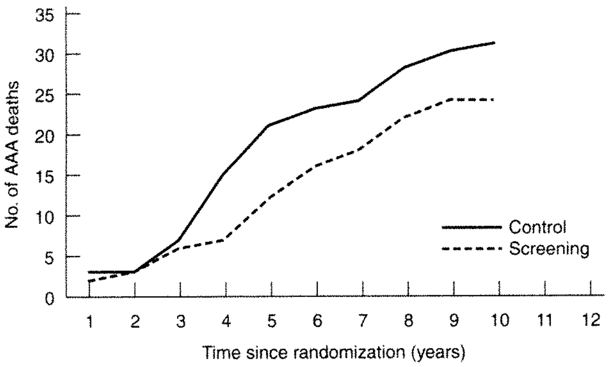

Fig. 2 Cumulative mortality from abdominal aortic aneurysm (AAA) by time since randomization

Table 2 Outcome for men with an abdominal aortic aneurysm who reached the criteria for elective surgery in the screening arm of the study

	No. of men	Operation for AAA
Deemed unfit for surgery	18	2
Declined outpatient appointment	2	0
Declined surgery	5	2
Too old for surgery	2	0
Returned to screening group	15	0
Planned surgery*	37	37
AAA ruptured before outpatient appointment	3	0
Total	82	41

*Elective and symptomatic. AAA, abdominal aortic aneurysm

Table 1 Details of abdominal aortic aneurysm surgery in control and study groups

		Study group (n = 49)			
			Complied with screening		
Operation type	Control group (n = 33)	Refused initial scan	Not visualized	Normal aorta	Recall group
Elective	17	3	1	0	32
Symptomatic	1	0	0	0	7
Emergency	13	2	0	0	4
Other	2	0	0	0	0
Total	33	5	1	0	43

however, an opportunity to assess the impact of the intervention over 10 years. Although these data should be interpreted with caution, they justify the larger, ongoing Multicentre Aneurysm Screening Study (MASS) that should report results in 2003. This 10-year follow-up suggested that the mortality benefit of ultrasonographic screening was maintained over time.

An important fact highlighted by these data is the need for a screening strategy to be acceptable to the target population. The benefits of even the most perfect screening programme will not be realized if individuals do not attend. Eighteen of 24 AAA deaths in the study group were in men who did not comply, either to the initial invitation to attend screening, to follow-up or to surgery. With greater awareness of the condition attendance rates may be expected to rise, thus reducing the mortality rate. Breast cancer screening clinics generally achieve compliance rates of between 75 and 85 per cent[2]. This may be a result of greater awareness of the disease and of the possible benefits of earlier identification that screening brings.

The remaining six AAA deaths in the study group were among compliers. This highlights the fact that the term 'screening' may be inappropriate as no preventative strategy has the ability to identify all disease and prevent all deaths. The current trend is towards describing screening programmes as 'risk reduction strategies' as people may have unrealistic expectations about what compliance to a programme can achieve.

The debate over the value of an AAA screening programme focused on the expected high elective surgical mortality rates in this elderly and relatively unhealthy population[3,4]. None of the six men in the compliant screening arm died as a result of elective surgery; three died with no surgery and three died following emergency surgery, illustrating that no screening programme can detect all subsequent cases.

A relatively large proportion of operations in the control group (17 of 33) were elective compared with 36 of 49 in the study group. The large amount of surgical activity in the unscreened population may have diluted the benefit of identifying an aneurysm earlier, before it presented clinically. A higher proportion of operations were done as an emergency in the control group (13 of 33 *versus* six of 49) but consideration of the absolute numbers of operations suggests that a screening programme may not reduce the emergency operation burden substantially. There remains a significant amount of emergency operation of screen-detected AAAs, largely related to refusal.

The present study did not ask GPs to exclude patients before randomization, but appointments were not offered to patients with significant co-morbidities. Despite this, 18 of 82 men with an expanding AAA were considered unfit for surgery and two were thought to be too old. In addition, two declined an appointment with the consultant to discuss treatment and five refused the operation when offered. This suggests that a clearer exclusion policy may have prevented unnecessary ultrasound scans. Perhaps the nature of the final intervention should be made clearer at the outset, to allow more informed choice on whether to enter the programme.

The possibility of bias needs to be examined when interpreting these data. ONS flagging was used to track all participants in the study and so all deaths registered as AAA have been added to the database. However, death may not be ascribed to AAA if rupture occurs outside of hospital and the cause of death is not confirmed by autopsy. This was more likely in the control group. It is more likely that sudden death will be certified as due to an AAA if the registering physician is aware of the patient's condition. This suggests that any mortality reduction reported from a suitably powered randomized trial will be conservative.

Death from AAA accounted for 2 per cent (55 of 2545) of all deaths reported over the 10-year follow-up interval of this study. This figure is in line with national AAA death rates. Even if the maximum reduction in AAA mortality rate reported here (52 per cent at 4 years) is realized by a screening programme, differences in all-cause mortality will be too small to be detectable in national statistics.

The results of the MASS trial should provide a more precise estimate of the reduction in AAA mortality rate that can be achieved through a screening programme. The results of the present study could be used to inform the debate about construction of an effective national programme to reduce the risk of dying from an AAA.

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Original article

New insight into bile flow dynamics in anomalous arrangement of the pancreaticobiliary duct

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Background: In an anomalous arrangement of the pancreaticobiliary duct (AAPBD), two-way regurgitation, including pancreaticobiliary reflux and biliopancreatic reflux (BPR), might occur. Although pancreaticobiliary reflux has been shown by high levels of pancreatic enzymes in bile, there have been few reports of clinical evidence of BPR in AAPBD. Recently, spiral computed tomography combined with intravenous infusion cholangiography (IVC–SCT) has been reported to be useful for visualizing the biliary anatomy, which may allow a new insight into bile flow dynamics in AAPBD.

Methods: Fifteen paediatric patients with AAPBD were examined before operation using both IVC–SCT and endoscopic retrograde cholangiopancreatography. Visualization of the main pancreatic duct on IVC–SCT defined the presence of BPR. Blood samples were also analysed when the initial symptom was strongly present.

Results: BPR was observed in six of 15 patients. Pancreatic enlargement and hyperamylasaemia were significantly more frequent in patients without BPR than in those with BPR. Levels of enzyme markers of cholestasis were also higher in the former group.

Conclusion: The present study, using IVC–SCT, has identified clinical evidence of BPR in AAPBD. Further studies are necessary to determine the correlation between BPR and clinical manifestations.

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Introduction

Anomalous arrangement of the pancreaticobiliary duct (AAPBD) is a congenital biliary anomaly that is commonly associated with congenital biliary dilatation, and is defined as an anatomical maljunction of the bile duct and the pancreatic duct outside the duodenal wall[1,2]. In AAPBD, freed from the influence of the sphincter of Oddi, pancreatic juice can regurgitate into the biliary tree and bile juice can also enter the pancreatic duct. These regurgitations are considered to be responsible for the clinical symptoms of AAPBD, including cholestasis or abdominal pain with hyperamylasaemia. Previous clinical studies have shown higher levels of pancreatic enzymes such as amylase, lipase and phospholipase A_2 in bile in patients with AAPBD[3–5], which are indicative of pancreaticobiliary reflux. However, there have been few clinical reports of evidence of the reverse reflux, biliopancreatic reflux (BPR), in AAPBD[6].

Recently, spiral computed tomography combined with intravenous infusion cholangiography (IVC–SCT) has been reported to be useful in biliary surgery, because it shows more anatomical details of the biliary tract than conventional intravenous infusion cholangiography[7,8]. Bile duct imaging by this method may reflect the actual bile flow in various pathophysiological conditions.

In this study, IVC–SCT was used in patients with AAPBD to diagnose BPR. The purpose of the study was to determine whether there was a correlation between BPR and its clinical manifestations in patients with AAPBD.

Patients and methods

From January 1997 to December 2000, 15 children with AAPBD were diagnosed and treated. There were 11 girls and four boys, of mean(s.e.m.) age 5·3(4·3) (range 1–17) years. The initial presentation was abdominal pain in 12 patients, jaundice in three and an abdominal mass in one. One patient had haematuria and was diagnosed incidentally with AAPBD. Blood samples were obtained from all patients to determine liver function and serum amylase levels at presentation. Hyperamylasaemia (more than 200 units/l) was present in seven patients. Of these, only one patient had pancreatic duct dilatation and was diagnosed with acute pancreatitis.

The diagnosis of AAPBD was mainly made by preoperative endoscopic retrograde cholangiopancreatography (ERCP) under general anaesthesia. The diagnostic criteria for AAPBD were (1) the presence of a markedly long common channel of the common bile duct and the major pancreatic duct over 10 mm in length and (2) an apparently anomalous junction of the common bile duct and the major pancreatic duct[9]. AAPBD was classified into two types (*Fig. 1*): the P-C type, in which the major pancreatic duct joins the common bile duct, and the C-P type, in which the common bile duct joins the major pancreatic duct[10]. In this study, nine patients had the P-C type of AAPBD and 6 had the C-P type. Choledochal dilatation was noted in 14 of the 15 patients with AAPBD (eight with the cystic type and six with the fusiform type).

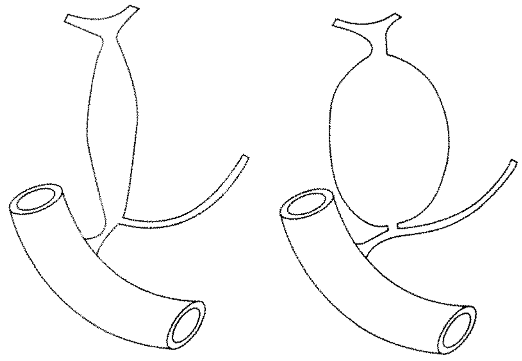
Excision of the gallbladder and the extrahepatic bile duct followed by Roux-en-Y hepaticojejunostomy was performed in all 15 patients. There was no evidence of pancreatic inflammation at surgery in any patient. Amylase levels in the bile of the gallbladder and the common bile duct were analysed using bile specimens obtained intraoperatively.

All patients were examined before operation using IVC–SCT after the initial symptom had subsided completely with conservative therapy. Briefly, after fasting for at least 4 h, meglumine iotroxate (Biliscopin® 50 mg/ml; Schering, Berlin, Germany) 1–3 ml/kg was administered intravenously over 30 min before SCT, and adverse reactions were carefully recorded. About 20 min after injection, SCT was performed with a table feed of 5 mm/s, a pitch of 1·0 (120 kV (peak) and 50–100 mA) in Asteion VR™ (Toshiba Medical, Tokyo, Japan). Sedation with chloral hydrate was used if necessary. The imaging results were assessed by two authors (K.T. and S.F.). Partial visualization of the main pancreatic duct on the film defined the presence of BPR (*Fig. 2*). The ratio of the largest width of the pancreas head to the width of the vertebral body on the same film was calculated in order to evaluate the size of the pancreas[11]. The widest transverse diameter of the common bile duct, and the main pancreatic duct diameter on the ERCP film were also measured. The main pancreatic duct was considered to be dilated if it was over 2 mm in diameter[12]. The study was approved by the Medical Committee on Human Studies of this hospital, and written informed consent was obtained from the parents of all patients.

All values are expressed as mean(s.e.m.). Statistical comparison was performed using Student's t test, Mann–Whitney two-sample test and Fisher's exact probability test. $P < 0{\cdot}05$ was considered significant.

Results

IVC–SCT was performed in all 15 patients without any adverse reaction. BPR was noted in six of the 15 patients. The clinical features of patients with ($n = 6$) and without ($n = 9$) BPR were compared (*Table 1*). There were no



a P-C type **b** C-P type

Fig. 1 Anomalous arrangements of the pancreaticobiliary duct. **a** In the P-C type, the major pancreatic duct joins the common bile duct. **b** In the C-P type, the common bile duct joins the major pancreatic duct

Table 1 Comparison of clinical features in patients with and without biliopancreatic reflux in the presence of an anomalous arrangement of the pancreaticobiliary duct

	BPR present ($n = 6$)	BPR absent ($n = 9$)
Age (years)*	7·3 (3–17)	3·9 (1–8)
Sex ratio (F : M)	4 : 2	7 : 2
Initial symptom		
Abdominal pain	4	8
Jaundice	0	3
Abdominal mass	1	0
Incidental	1	0
Hyperamylasaemia	1	6†
Form of choledochal dilatation		
Cystic	4	4
Fusiform	2	4
No dilatation	0	1
Type of AAPBD		
P-C	5	4
C-P	1	5

*Values are mean (range). BPR, biliopancreatic reflux; AAPBD, anomalous arrangement of the pancreaticobiliary duct. †$P = 0{\cdot}03$ *versus* BPR present (Fisher's exact test) significant differences in age, gender, initial symptoms and forms of choledochal dilatation between the two groups. Hyperamylasaemia was significantly more common in patients without BPR than in those with BPR ($P = 0{\cdot}03$). The patient with acute pancreatitis did not have BPR. The P-C type of AAPBD was found in five of the six patients who had BPR, and in four of the nine children in whom BPR was not present.

Table 2 shows a comparison of the radiographic and laboratory data between the two groups. There was no significant difference in the mean common bile duct diameter. The mean value of the pancreas-to-vertebra ratio was significantly higher in patients without BPR ($P < 0{\cdot}01$).

There was no significant difference in liver function tests between the two groups, apart from a higher level of leucine aminopeptidase in the BPR absent group. Serum amylase levels had no significant difference between the two groups. Although amylase levels in the bile extracted from the common bile duct and the gallbladder were markedly raised, there was no significant difference between the two groups.

Discussion

In AAPBD it seems that two-way regurgitation including pancreaticobiliary reflux and BPR may occur. Pancreaticobiliary reflux has been confirmed by the higher concentration of pancreatic enzymes in the bile of patients with AAPBD[3–5]. As noted in previous reports, regurgitation of pancreatic juice into the biliary tract may induce acute or chronic inflammation of the bile duct, which is supported by histopathological studies of the biliary tract[13]. On the other hand, there has been little clinicopathological evidence of BPR in AAPBD, since it is difficult to evaluate the dynamics of bile flow. There have been some reports describing pancreatic duct reflux during operative cholangiography for patients with biliary disease[14,15]. However, most patients examined in these studies had gallstones without AAPBD. In addition, this imaging technique does not necessarily represent the physiological state of the pancreaticobiliary system because the intraluminal pressure of the pancreaticobiliary duct is increased by the injection of contrast.

Recently, IVC–SCT has been reported to be useful for preoperative biliary investigation. The great advantage of

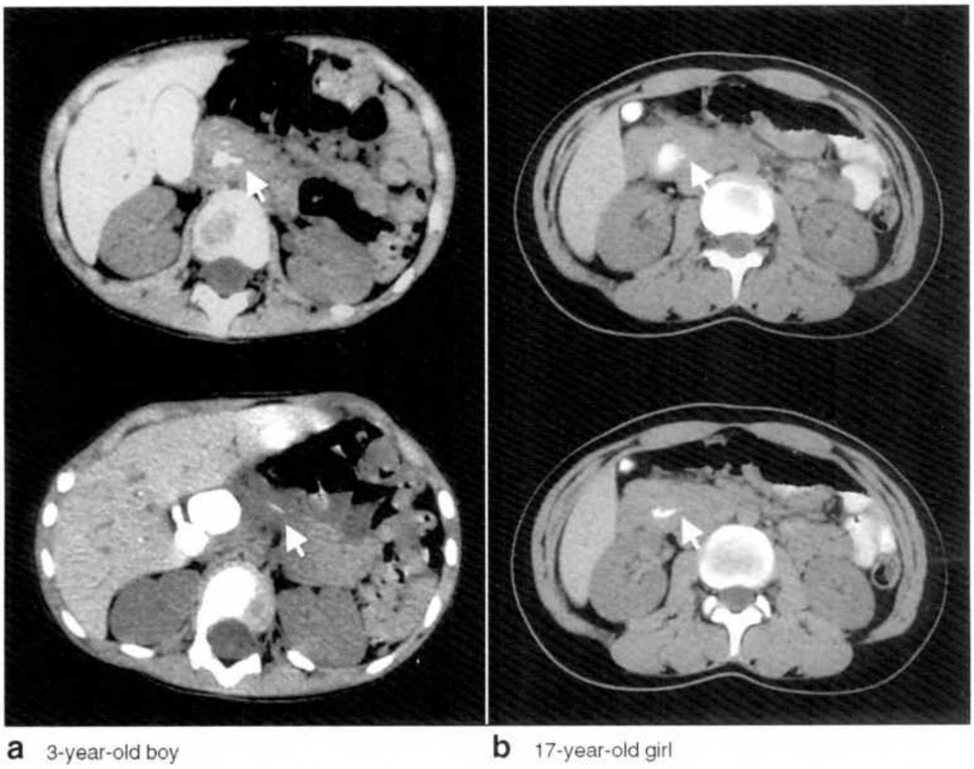

a 3-year-old boy **b** 17-year-old girl

Fig. 2 Biliopancreatic reflux on spiral computed tomography combined with intravenous infusion cholangiography in two patients with an anomalous arrangement of the pancreaticobiliary duct. The main pancreatic duct is shown by arrows. **a** A 3-year-old boy with fusiform dilatation of the common bile duct. **b** A 17-year-old girl with cystic dilatation of the common bile duct

Table 2 Comparison of radiographic and biochemical values in patients with and without biliopancreatic reflux

	BPR present ($n = 6$)	BPR absent ($n = 9$)	P*
Diameter of the common bile duct (mm)	35·3(5·9)	22·9(3·1)	0·06*
Dilatation of the main pancreatic duct	4	3	0·20†
Pancreas-to-vertebra ratio	0·65(0·03)	0·80(0·05)	< 0·01*
Serum amylase (units/l)	346·8(221·6)	617·4(261·5)	0·24‡
Aspartate aminotransferase (units/l)	219·8(115·0)	201·8(50·4)	0·48‡
Alanine aminotransferase (units/l)	143·2(73·4)	242·3(48·6)	0·44‡
Total bilirubin (mg/dl)	0·97(0·17)	2·47(0·69)	0·05‡
Direct bilirubin (mg/dl)	0·31(0·13)	1·38(0·51)	0·19‡
Alkaline phosphatase (units/l)	810·8(243·3)	2 950·1(1614·5)	0·06‡
γ-glutamyltranspeptidase (units/l)	240·0(166·0)	515·0(194·6)	0·16‡
Leucine aminopeptidase (units/l)	85·8(21·6)	341·6(87·9)	0·04‡
Amylase level in the common bile duct (units/l)	55 336(21 970)	86 734(45 068)	0·81‡
Amylase level in the gallbladder (units/l)	83 219(36 802)	118 405(46 685)	0·60*

Values are mean(s.e.m.). BPR, biliopancreatic reflux. *Student's t test; †Fisher's exact test; ‡Mann–Whitney two-sample test

this modality is its ability to produce high-quality images without respiratory artifacts and provide more anatomical detail of the biliary tract than conventional intravenous infusion cholangiography[7,8]. Contrast materials in IVC–SCT are absorbed by the hepatocytes and excreted into the bile after intravenous injection. Biliary visualization is based on these pharmacokinetics[16]. Therefore, IVC–SCT is considered to reflect more dynamic and physiological bile flow than operative cholangiography.

In this study, six of 15 patients with AAPBD showed BPR. Hyperamylasemia was observed more frequently in patients in whom BPR was absent. It is hypothesized that hyperamylasaemia in patients with AAPBD is caused by cholangiovenous reflux[17,18]. The present findings suggest that the regurgitation of pancreatic juice into the biliary system may induce hyperamylasaemia in patients who have AAPBD without BPR. Moreover, the trend towards higher levels of the marker enzymes of cholestasis in patients without BPR suggests that bile drainage from the bile duct into the duodenum may be smoother in patients with BPR than in those without BPR.

The pancreas-to-vertebra ratio was significantly higher in patients who did not show BPR. Although it remains to be confirmed whether pancreatic enlargement in SCT relates to acute pancreatitis, it is notable that one patient without BPR had acute pancreatitis. Further investigation is necessary to determine the correlation between acute pancreatitis and pancreas swelling.

A positive pressure gradient from the pancreatic duct to the bile duct has been reported in patients without AAPBD[19]. The sphincter mechanism is disturbed in patients with AAPBD, which allows two-way regurgitation into the pancreaticobiliary duct, and this study revealed that BPR could occur against the pressure gradient of the pancreaticobiliary system in some patients with AAPBD. Although further studies are necessary to determine the correlation between BPR and any clinical manifestations, these findings could be useful in further elucidating the pathophysiology and bring new insights into the bile flow dynamics of the pancreaticobiliary system in patients with AAPBD.

Acknowledgements

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Original article

Transoesophageal echocardiography shows high risk of gas embolism during laparoscopic hepatic resection under carbon dioxide pneumoperitoneum

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Background: The ultrasonically activated scalpel (UAS) enables safe and effective laparoscopic tissue dissection, making hepatic resection feasible. This study compared blood loss and risk of gas embolism using the UAS during open hepatic resection and laparoscopic hepatic resection.

Methods: Female pigs were divided into two groups for laparoscopic ($n = 7$) and open ($n = 5$) left hepatic lobectomy. The UAS was used for both tissue cutting and coagulation. Laparoscopic liver resection was performed under carbon dioxide pneumoperitoneum (intraperitoneal pressure 12 mmHg). During surgery animals were monitored haemodynamically by an arterial line and Swan-Ganz catheter. Two-dimensional transoesophageal echocardiography (2D-TEE) was used to detect gas emboli with special attention to the right atrium and ventricle. Gas emboli were graded according to size, and correlated with haemodynamic and blood gas data.

Results: During open and laparoscopic hepatic resection the UAS resulted in minimal blood loss and effective tissue dissection. No air embolism was seen during open surgery. With laparoscopic hepatic resection 2D-TEE revealed gas embolism in all animals. Gas embolism was accompanied by cardiac arrhythmia in four of seven animals. No direct correlation was observed between embolism episodes and blood gas variables. There were no deaths after episodes of embolization. A significant decrease in arterial partial pressure of oxygen was seen at the end of the laparoscopic procedure in all animals.

Conclusion: The UAS causes minimal blood loss during both open and laparoscopic hepatic resection. Laparoscopic liver dissection under carbon dioxide pneumoperitoneum carries a high risk of gas embolism.

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Introduction

Laparoscopic surgery has rapidly become an extensively used technique. Besides cholecystectomy and operations on the gastrointestinal tract, resection of solid organs such as the adrenal glands, kidneys and spleen has been performed[1–4]. Laparoscopic partial hepatectomy, however, presents unique anatomical difficulties and surgical challenges. A major problem is haemostasis in the plane of transection. The management of haemorrhage from large intrahepatic vessels or deep dissection, which is necessary to maintain visual control of the operating field, is sometimes difficult. In the past few years new laparoscopic instruments that apply novel energy forms have decreased the risk of operative blood loss and made effective laparoscopic hepatic dissection feasible[5–11].

Carbon dioxide embolization during laparoscopic partial hepatectomy, as well as in other laparoscopic procedures, is a potentially lethal complication[12–17]. The most likely aetiology, aside from accidental placement of the Veress needle into a vessel during initial insufflation, is venous laceration during laparoscopic dissection. Because of the specific hepatic anatomy, the risk of gas embolism after venous injury while dissecting the liver parenchyma under a pneumoperitoneum is thought to be enhanced. This hypothesis, however, has never been precisely tested.

In this experimental study the safety of tissue cutting and coagulation using an ultrasonically activated scalpel (UAS) was compared in open *versus* laparoscopic liver resection, along with an evaluation of gas pulmonary embolization.

Materials and methods

Female Troll pigs ($n = 12$; 81–92 kg) were maintained under standardized conditions and received humane care in compliance with the institution's guidelines and according to veterinary law. They were deprived of food overnight but allowed free access to water before surgery. Animals were divided into two groups for laparoscopic ($n = 7$) and open ($n = 5$) left lateral hepatic resection. General anaesthesia was induced with intramuscular ketamine 20 mg/kg and xylazine 4 mg/kg followed by intravenous etomidate 0·2 mg/kg and fentanyl 0·02 mg/kg. After tracheotomy animals were intubated and mechanically ventilated with a positive end-expiratory pressure (5 cmH_2O) at a tidal volume of 15 ml/kg using a mixture of oxygen and air (fraction of inspired oxygen 0·6). The respiratory rate was adjusted to achieve an end-tidal carbon dioxide level of 20–35 mmHg, after which minute ventilation remained fixed. Pancuronium bromide 0·1 mg/kg was given for neuromuscular paralysis and repeated every 45 min.

The right carotid artery and the right internal jugular vein were surgically exposed and cannulated (16-G), and a flow-directed pulmonary arterial Swan-Ganz catheter (Baxter, Irvine, California, USA) was placed in the pulmonary artery. All animals were monitored and haemodynamic variables (cardiac output (CO), heart rate (HR), mean arterial blood pressure (MAP), central venous pressure (CVP), pulmonary arterial pressure (PAP), pulmonary wedge pressure (PWP), arterial partial pressure of oxygen (pO_2), arterial partial pressure of carbon dioxide (pCO_2)) and blood gas variables were recorded repeatedly. A two-dimensional transoesophageal echocardiography (2D-TEE) probe (5–7 MHz multiplane TEE probe, Vingmed System 5, GE, Horten, Norway) was inserted via an oesophagotomy with special attention to the right atrium (RA), right ventricle (RV) and right ventricular outflow tract (RVOT). Gas bubbles were defined as echogenic particles that moved with blood flow. Gas emboli were graded according to size by TEE (*Table 1*), and correlated with haemodynamic and blood gas data. All TEE images were videotaped and correlated with surgical events. Videotaped TEE images were evaluated after operation twice (by two investigators).

In the laparoscopy group a minimal open laparotomy was performed in the central mid-abdomen to insert the laparoscope and create the carbon dioxide pneumoperitoneum (intraperitoneal pressure 12 mmHg). The pig was positioned semilaterally and three more 10-mm ports (Ethicon, Cincinnati, Ohio, USA) were inserted subcostally on the anterior axillary line, the midclavicular line, and in the epigastrum for left lateral liver resection. Before hepatic dissection the CVP was adjusted to 12 mmHg (according to the intraperitoneal pressure) and maintained during the resection. Parenchymal dissection of the left lateral hepatic lobe was performed with the UAS (UltraCision™, Smithfield, Rhode Island, USA). No vascular inflow occlusion was performed. Larger bile ducts (diameter more than 2–3 mm) and branches of portal or hepatic veins were clipped. The resected specimen was placed in a nylon bag and delivered via an extension of the anterior axillary incision. In the open group a transverse abdominal incision was performed and left lateral lobectomy of the liver was carried out as described above. The resected surface of the liver was observed for at least 30 min to document the efficacy of haemostasis. At the end of the surgical procedure the blood loss during resection was measured in the suction bag followed by lethal intracardiac injection of saturated potassium chloride solution.

Table 1 Staging of gas embolism detected by two-dimensional transoesophageal echocardiography

Stage	Transoesophageal echocardiography
0	No emboli in RA, RV and RVOT
I	Single gas bubbles in RA, RV and RVOT
II	Gas emboli filling less than half the diameter of RA, RV and RVOT
III	Gas emboli filling more than half the diameter of RA, RV and RVOT
IV	Gas emboli completely filling the diameter of RA, RV and RVOT

RA, right atrium; RV, right ventricle; RVOT, right ventricular outflow tract

Data were expressed as mean(s.d.). The Mann–Whitney *U* test and Fisher's exact test were used to determine statistical differences between the groups. $P < 0.05$ was considered significant.

Results

In open left lateral lobectomy of the liver the exclusive application of an UAS enabled effective tissue dissection with minimal blood loss. No additional method was needed for haemostasis, even from a deeper transection plane. Haemodynamic and blood gas variables were stable throughout the intervention. There were no episodes of cardiac arrhythmia in the open group. During the surgical procedure no air embolism was detected by 2D-TEE.

During laparoscopic liver resection gas embolism was detected echocardiographically (*Fig. 1*) in all animals ($P = 0.001$ *versus* open surgery, Fisher's exact test).

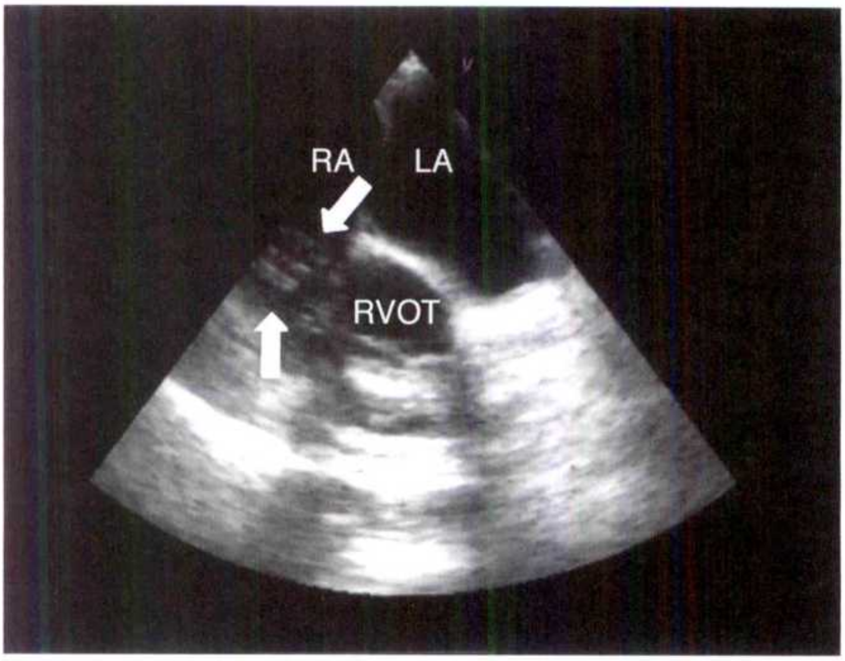

Fig. 1 Two-dimensional transoesophageal echocardiography showing the right atrium (RA), left atrium (LA) and right ventricular outflow tract (RVOT). Arrows indicate air entering the RVOT

Episodes of embolism ranged from seven up to 20 per procedure. Movement of carbon dioxide emboli from the RA to the RV was easily seen. The detected emboli were classified entirely as stage I (56 per cent) or II (44 per cent), i.e. all emboli were smaller than half the diameter of the RA or RV. The right heart cavities cleared all carbon dioxide emboli within seconds; no carbon dioxide emboli were noted in the left heart cavities. In four of seven animals at least three episodes of gas embolism were accompanied by cardiac arrhythmias. In each case the arrhythmia was short lived and resolved spontaneously, without need for medical intervention. Tachycardiac arrhythmias predominated and were characterized by frequencies of between 180 and 250 beats per min. In one case a bradycardiac arrhythmia (35 beats per min) was seen. The corresponding gas emboli were classified as stage II. Cardiac arrhythmias were observed only during episodes of gas embolism. 'Gas lock', meaning acute cardiocirculatory failure with ineffective contraction of the cardiac chambers due to fulminant pulmonary embolism, was not observed. Embolism did not occur during creation of the pneumoperitoneum. The CVP was increased to correspond with the intraperitoneal pressure of 12 mmHg. Episodes of embolism were detected with the CVP above and below the intraperitoneal pressure.

No correlation between episodes of embolism and blood gas variables could be established. At the beginning of the laparoscopic procedure and the creation of carbon dioxide pneumoperitoneum there was a significant increase ($P < 0.05$) in pCO_2 (*Fig. 2*) and PWP, accompanied by a significant decrease in pH and CO. PAP increased, but changes were not statistically significant. MAP and HR did not change (*Table 2*). No immediate changes in any of the

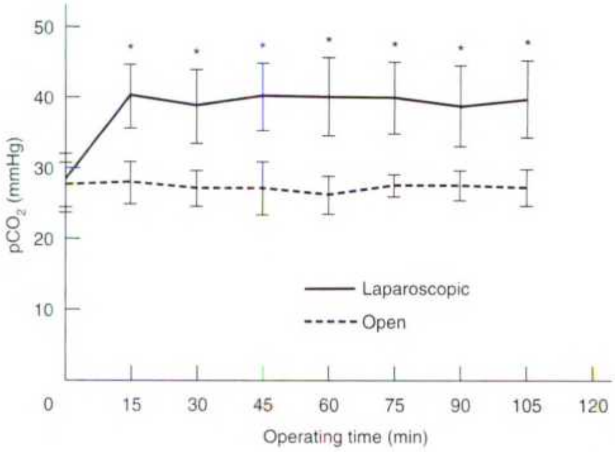

Fig. 2 Arterial partial pressure of carbon dioxide (pCO_2) during the course of laparoscopic (pneumoperitoneum created at 0 min) and open liver resection. Values are mean(s.d.). $^*P < 0.05$ *versus* start of laparoscopic operation and *versus* open group at same timepoint (Mann–Whitney *U* test)

Table 2 Barometric and haemodynamic effects of laparoscopic and open liver lobectomy in pigs at the start of the operation and after 90 min

	Laparoscopic resection (n = 7)		Open resection (n = 5)	
	Start	90 min	Start	90 min
Arterial partial pressure of carbon dioxide (mmHg)	27·9(4·1)	38·8(5·7)*	27·7(3·1)	27·5(2·1)
Arterial partial pressure of oxygen (mmHg)	233(24)	137(31)*	246(22)	238(21)
pH	7·4(0·1)	7·3(0·1)*	7·5(0·1)	7·4(0·1)
Cardiac output	2·6(0·8)	1·5(0·7)*	2·6(0·8)	2·5(0·9)
Mean arterial pressure (mmHg)	86·1(18·3)	83·7(24·1)	84·4(22·4)	78·2(28·2)
Mean pulmonary arterial pressure (mmHg)	27·7(3·1)	30·4(5·3)	26·4(5·2)	27·6(6·5)
Pulmonary wedge pressure (mmHg)	11·1(2·1)	15·9(2·2)*	11·8(3·3)	10·5(2·8)
Central venous pressure (mmHg)	10·4(3·6)	11·5(3·2)	10·1(4·1)	9·1(3·6)
Heart rate (beats/min)	103(29)	108(31)	105(23)	106(34)

Values are mean(s.d.). Animals in laparoscopic group had pneumoperitoneum and intraperitoneal pressure of 12 mmHg. *$P < 0·05$ *versus* start of laparoscopic resection and *versus* open resection at 90 min (Mann–Whitney U test)

above variables correlated directly with an episode of embolism. As the carbon dioxide pneumoperitoneum was created and during the course of laparoscopic left lateral liver lobectomy, there was a significant linear decrease in pO_2 (*Fig. 3*). No immediate change in pO_2 occurred with any episode of gas embolism. Investigation of videotaped TEE images revealed that gas embolism took place throughout all phases of the dissection but was observed predominantly with surgical manoeuvring in a deeper plane of parenchymal transection. The exclusive application of an UAS during the laparoscopic procedure, as in the open procedure, enabled effective tissue dissection with little blood loss. No additional thermic method was required to guarantee haemostasis, even from a deeper plane of transection. Control of haemorrhage was more difficult to achieve with laparoscopic than open liver resection and it was also more difficult to achieve in deeper planes of transection. The operative time in the laparoscopic lobectomy group (90(14) min) was significantly longer than that in the open group (61(8) min). There were no significant differences in intraoperative blood loss (168(55) *versus* 186(62) ml for laparoscopic *versus* open operation respectively), haemoglobin levels or perioperative complications.

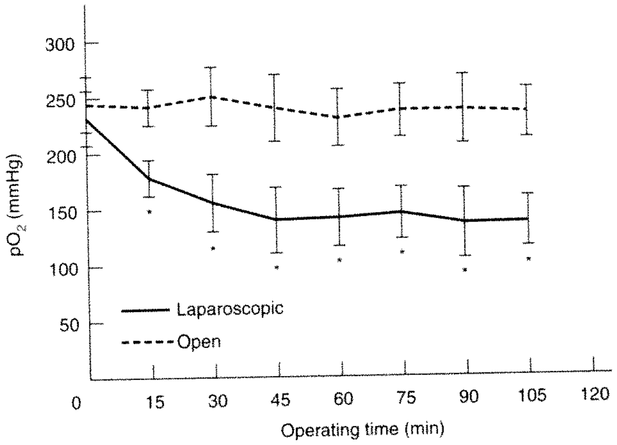

Fig. 3 Arterial partial pressure of oxygen (pO_2) during the course of laparoscopic (pneumoperitoneum created at 0 min) and open liver resection. Values are mean(s.d.). *$P < 0·05$ *versus* start of laparoscopic operation and *versus* open group at same timepoint (Mann–Whitney U test)

Discussion

With the development and improvement of laparoscopic instruments that use novel energy forms for tissue dissection, the spectrum of liver disease in which laparoscopic intervention can be performed safely has been extended[8,11,15,18–24]. For patients with hepatic malignancy accompanied by reduced liver function, laparoscopic surgery may result in a better operative outcome than open surgery. Moreover, the laparoscopic procedure allows liver resection in patients with advanced cirrhosis and evidence of liver failure, in whom open surgery might be contraindicated[25,26]. Although the reduction in intraoperative trauma results in faster postoperative recovery of the patient, haemostasis and embolism are two problematic areas associated with laparoscopic liver surgery.

In this study an UAS for laparoscopic left liver lobectomy enabled effective tissue dissection with minimal blood loss. No significant differences were found between intraoperative blood loss or haemoglobin levels during laparoscopic and open surgery. Control of haemorrhage was more difficult during laparoscopic than open surgery owing to the lack of tactile response and the limitations of exposure. Hand-assisted laparoscopic liver surgery might help to overcome this problem by facilitating effective retraction of the liver parenchyma, thereby improving visual control of the operative field[27,28]. It might also simplify the management of major intraoperative complications such as bleeding from a deeper plane of transection. To prevent major bleeding, clamping of the portal triad is now widely used[25,26,29,30]. This could be difficult when performed laparoscopically and hand assistance might facilitate the procedure. Clamping the portal triad during a pneumoperitoneum could have a significant impact on haemodynamics, causing deterioration of important cardiovascular variables (e.g. CO, MAP)[31].

In this study venous gas embolization occurred in all animals during laparoscopic liver resection under carbon dioxide pneumoperitoneum. Using 2D-TEE, which is the most sensitive method for the detection of venous emboli[32–35], between seven and 20 embolic episodes per animal were detected. These emboli were smaller than half the diameter of the RA or RV but, besides short reversible cardiac arrhythmia, no direct correlation between gas embolization and cardiorespiratory events was seen.

Pathophysiological effects of gas embolism are due to the transient accumulation of gas in the pulmonary arterioles. Besides the nature of the gas, the consequences depend on the volume as well as the rate of gas entry[36]. Slow infusion of air is known to occlude the pulmonary circulation, and to cause an instant increase in PAP and a progressive decrease in peripheral resistance. This may be compensated for by an increase in CO, which when it fails will result in hypotension. Intravenous injection of a large air bolus instead can produce an 'air lock', meaning complete mechanical obstruction of the right heart cavities causing acute hypotension or even heart arrest[37].

Venous embolism of carbon dioxide, the gas used predominantly in laparoscopic surgery, is known to cause fewer haemodynamic changes than air or oxygen[38,39], owing to the high solubility of carbon dioxide in blood. The clinical impact of carbon dioxide embolism during laparoscopy might therefore be limited and the postoperative course relatively benign[40]. Continuous intravenous insufflation of carbon dioxide can cause diffusion of other gases (mainly oxygen) into the carbon dioxide emboli[41], which could significantly extend the time needed for embolism absorption and may be responsible for the permanent injuries and deaths reported after carbon dioxide embolism[13,14,42]. Acute hypoxaemia paralleled by hypocapnia, as is seen in fulminant air embolism during open surgery[43,44], is rare in carbon dioxide embolization during pneumoperitoneum. Moreover, the pneumoperitoneum interferes with the potential haemodynamic effects due to carbon dioxide embolism, complicating the clinical diagnosis.

The significant decrease in pO_2 during the early course of laparoscopic liver lobectomy is thought to be caused by the creation of pneumoperitoneum. No immediate change in pO_2 due to a single episode of gas embolism was observed. Whether the decrease in pO_2 might have been reinforced by relapsing episodes of gas embolism remains speculative. Neurological damage due to a paradoxical gas embolism could not be evaluated since the animals were killed before regaining consciousness. In patients with advanced chronic liver disease direct intrapulmonary arteriovenous shunting is not uncommon. Hopkins *et al.*[45] reported that 47 per cent of patients with cirrhosis had right-to-left shunts. The finding that the majority of embolic episodes are not clinically significant is consistent with results of other studies that used TEE to detect gas emboli during laparoscopic surgery[32,46,47]. To produce a significant haemodynamic effect, detectable by means other than TEE, a large amount of carbon dioxide must enter the venous circulation[32]. Such severe gas embolism is rare. This study does, however, reveal that episodes of minor gas embolism during laparoscopic hepatic resection occur surprisingly often with a carbon dioxide pneumoperitoneum.

To reduce the risk of carbon dioxide embolism, an increase in CVP to a level permanently exceeding the intraperitoneal pressure may seem appropriate. Intraoperative blood loss during liver resection, however, correlates directly with CVP[48]. Lowering CVP during open surgery is an effective way of reducing bleeding of the dissected liver, therefore improving morbidity and mortality rates[48–50]. Minimizing the risk of a potentially hazardous embolism means performing surgery without pneumoperitoneum. Mechanical systems that lift the abdominal wall[24,26,29,51–53] might solve this problem. This would avoid the risk of carbon dioxide embolism (but not that of air embolism) and reduce adverse haemodynamic and respiratory effects caused by conventional pneumoperitoneum. The physiological benefit is achieved at the expense of surgical exposure, which might increase the difficulty of haemorrhage management. A combination of low-pressure pneumoperitoneum with abdominal wall lifting systems, to provide good surgical exposure, may be a satisfactory solution[53].

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Original article

Complications and functional outcome following artificial anal sphincter implantation

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Background: The postoperative complications and functional outcome following 24 consecutive implantations of an artificial anal sphincter were assessed prospectively.

Methods: A total of 24 artificial anal sphincters (Acticon Neosphincter®) were implanted in 22 patients (mean age 47 years). The mean follow-up period was 28 (range 6–48) months. Results were assessed prospectively by two independent observers at 4-month intervals. The cumulative probability of artificial anal sphincter removal was analysed by the Kaplan–Meier method.

Results: Five patients were free of complications. During the postoperative period, complications occurred in nine patients, two of whom required reoperation. During follow-up, complications developed in ten patients, nine of whom were reoperated. Definitive device explantation was necessary in seven patients. The cumulative probability of device explantation was 44 per cent at 48 months. The 15 patients with functioning implants were followed up for a mean of 26 (range 7–48) months. Continence grading improved from a mean of 18 (range 14–20) in the preoperative period to 4 (range 0–14) after operation ($P < 0{\cdot}001$). Resting anal canal pressure in patients with a functioning implant increased from a mean of 35 (range 8–87) mmHg before operation to 54 (range 34–70) mmHg after implantation ($P < 0{\cdot}01$).

Conclusion: An artificial anal sphincter is a useful alternative for refractory faecal incontinence but the incidence of late postoperative complications is high.

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Introduction

A number of surgical procedures are available for treatment of patients with severe faecal incontinence when conventional surgical repair is not indicated or has failed[1]. Artificial anal sphincter implantation is a relatively recent procedure[2,3]. The Acticon Neosphincter® (American Medical Systems, Minneapolis, Minnesota, USA) artificial bowel sphincter has been developed from the artificial urinary sphincter. It consists of an inflatable cuff positioned around the upper anal canal, a pressure-regulating balloon that is placed deep in the pelvis to maintain cuff closure, and a pump that is placed in the scrotum or labium to empty the cuff for defaecation. The Acticon® device has been tested in a limited number of European and North American centres, usually in a small number patients with varying follow-up intervals and inconclusive results[3–8]. To date, therefore, limited data regarding patient outcomes after surgical placement of the device are available. In this prospective study the complications and functional outcomes following implantation of the Acticon® device for severe anal incontinence are reported.

Patients and methods

Between November 1996 and January 2000, 22 patients (five men and 17 women) with a mean age of 47 (range 17–72) years with severe faecal incontinence underwent artificial bowel sphincter implantation. They had been incontinent for a mean period of 18 (range 2–39) years. Incontinence resulted from neuropathy ($n = 5$), anal atresia ($n = 3$), perineal trauma ($n = 3$), direct sphincter disruption from operative trauma (*n* = 4) or obstetric injury (*n* = 6), and Steinert's myotonic dystrophy (*n* = 1). All patients had previously been treated with conservative therapy (diet and medical treatment). Biofeedback was used in 12 patients and 17 patients had undergone previous surgical procedures in an attempt to alleviate their incontinence. These included perineal repair in eight patients, sphincteroplasty in five and graciloplasty in one. Two patients had a colostomy to manage their symptoms. All patients provided written informed consent after being advised of the experimental nature of the procedure. The study protocol was approved by the institutional review board of the participating hospitals.

Before operation the degree of incontinence was quantified using the Cleveland Clinic Florida 20-point incontinence scoring system[9]. This scale takes into account the frequency and type of incontinence (to flatus, liquid, solid stool; need to wear pads; and the impact of incontinence on lifestyle). Scores range from 0 (total continence) to 20 (the most severe incontinence). To obtain reliable data regarding faecal incontinence, each patient was required to complete the incontinence questionnaire daily over a month. The severity of preoperative faecal incontinence was not assessed in patients with a colostomy. Other investigations included anorectal manometry, pudendal nerve terminal motor latency measurement, and transanal ultrasonography with a 7-MHz transducer (B & K Medical, Gentofte, Denmark). The preoperative phase included complete colonic preparation and antibiotic and deep venous thrombosis prophylaxis. The technique for implantation of the Acticon Neosphincter® has been described previously[4]. Operations were performed by two colorectal surgeons (H.O. and J.V.R.). Activation and use of the device occurred at 8 weeks after healing of the wound. Pre-existing stomas were closed electively 2 months after activation of the device.

Postoperative results were assessed prospectively by two independent observers at 4-month intervals. The closing date of the study was 30 November 2000 to ensure a minimum follow-up of 6 months for the last patient included. The mean follow-up from the operation to the closing date was 28 (range 6–48) months. Implant removal and failure to accept implantation of another device was an exclusion criterion for follow-up assessment. Complications related to operations or to the implanted devices were recorded. In patients in whom an appropriate level of continence was not obtained, plain radiographs were taken to assess the correct alignment of the system. The efficacy of the procedure was evaluated after operation by improvement in the continence grading scale as recorded by the patient at each follow-up visit and anorectal manometric assessment.

Statistical analysis

χ^2 test (with Yates' correction when necessary), two-tailed Fisher's exact probability test and calculation of relative risks with 95 per cent confidence intervals were applied for comparison of complications and reoperations during follow-up. Preoperative and postoperative manometric data and severity of incontinence were compared with the Wilcoxon signed rank test. The cumulative probability of removal of the device was analysed by the Kaplan–Meier method.

Results

Complications during the immediate postoperative period or at follow-up occurred in all but five patients.

Postoperative complications

Nine patients had complications in the postoperative period. Perineal and abdominal haematoma in four patients and perineal wound dehiscence in three was treated successfully with conservative measures. The remaining two patients had perineal infection and explantation of the system was required.

Complications at follow-up

Ten patients developed a total of 11 complications, and ten surgical operations were required in nine patients. Refilling of the cuff was required in one patient at 28 months of follow-up. Migration of the pump at 3 months after implantation occurred in one patient and required reoperation. Three patients presented with perineal pain at 3, 12 and 15 months after operation. The first was treated successfully with analgesics, but explantation of the device was required in the remaining two patients. One patient presented with incontinence due to unbuttoning of the cuff at 10 and 12 months of follow-up; reoperation was required on two occasions, the first to rebutton the cuff and the second for explantation and placement of a new device. Five patients developed cutaneous erosion and exteriorization of the tubes or the cuff. A patient with exteriorization of the tubes at 34 months required reoperation, with debridement and suture of the wound. In four patients with exteriorization of the cuff at 4, 6, 6 and 46 months, explantation of the device was performed.

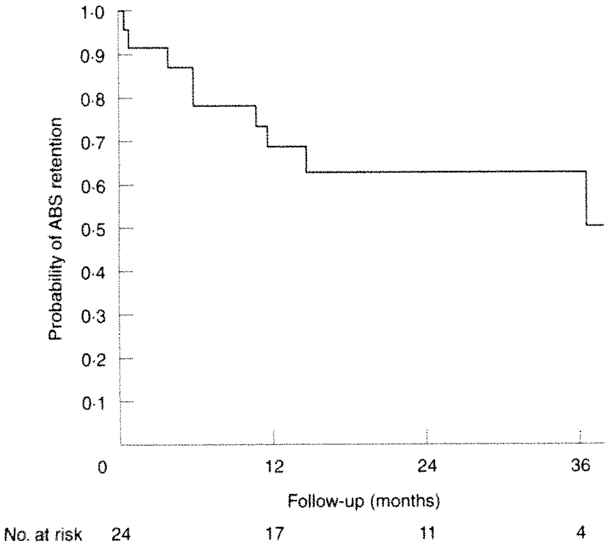

Fig. 1 Cumulative probability of device retention. ABS, artificial bowel sphincter

Explants and reimplants

Explantation of the device was necessary in eight patients. A new Acticon Neosphincter® was reimplanted in two patients, one of whom had perineal infection in the early postoperative period and another who had unbuttoning of the cuff at 12 months of follow-up. The latter patient finally presented with skin erosion and exteriorization of the cuff 6 months later and the device was explanted. Therefore, at the end of the study a total of 24 artificial anal sphincters had been implanted. Definitive explantation of the device was necessary on nine occasions in a total of seven patients. The cumulative probability of explantation of the device was 44 per cent at 48 months (*Fig. 1*).

There were no statistically significant differences in the number of complications that developed during the early postoperative period and at follow-up. However, the number of surgical reoperations carried out as a result of complications was significantly higher at follow-up than in the immediate postoperative period (relative risk 0·19 (95 per cent confidence interval 0·05 to 0·78); $P < 0{\cdot}005$).

Functional outcome

Functional outcome was assessed in the 15 patients with a functioning implant. The mean follow-up was 26 (range 7–48) months. Total continence was achieved in only four patients (*Table 1*). Fourteen patients achieved total continence for formed stool and only one patient experienced rare episodes of incontinence. However, several patients had problems related to continence of liquid stool and flatus, as a result of which four patients had to wear pads and two patients considered that these events caused alterations in their lifestyle. Continence scores improved from a mean of 18 (range 14–20) before implantation to 4 (range 0–14) after operation ($P < 0{\cdot}001$). Of the five patients with the worst results, the original diagnosis was anal atresia in three, Steinert's myotonic dystrophy in one and neuropathy with anal pain that required the use of analgesics in one.

Table 1 Functional outcome in 15 patients with a functioning implant (Cleveland Clinic Florida 20-point incontinence scoring system)

Incontinence type	Never	Rarely	Sometimes	Usually	Always
Solid (formed)	14	1			
Liquid	9	4	2		
Flatus	4		5	4	2
Wear pads	11			1	3
Lifestyle alteration	13		1	1	

Rarely, less than once per month; sometimes, less than once per week, once or more per month; usually, less than once per day, once or more per week; always, once or more per day

Two patients required laxatives and enemas owing to difficulties with evacuation. Resting anal pressure in patients with a functioning implant increased from 35 (range 8–87) mmHg before operation to 54 (range 34–70) mmHg after operation when the cuff was inflated ($P < 0{\cdot}01$).

Discussion

This study reports one of the two largest series[4] of patients with an Acticon Neosphincter® to date. As in previous published studies, none of the patients died. The causes of incontinence, duration of symptoms and number of patients who had previous unsuccessful surgery were similar to those reported by others[3–8].

The number of patients with minor postoperative complications recorded in this study was also similar to that reported in three previous studies[4–6]. However, the actual frequency of postoperative complications is difficult to ascertain. Lehur *et al.*[4] reported urinary tract infection as a complication, whereas postoperative haematoma and emptying difficulties were not taken into account. In contrast, the rate of urinary tract infection was not mentioned in the remaining reports[3,5–8]. Similarly, faecal impaction occurred in the early postoperative period in some studies[6,7] but was not mentioned in others[3–5,8].

The large number of serious complications that developed during follow-up was a striking finding of the present

Table 2 Complications, explants, reimplants and surgical reoperations in published literature

Reference	Year	No. of patients	Infection	Erosion*	Mechanical failure†	Explants	Reimplants	Patients with definitive explants	Early reoperation‡	Late reoperation§	No. of patients reoperated
Wong *et al.*[3]	1996	12	2	0	3	5	5	1	2	4	6
Lehur *et al.*[4]	2000	24	1	3	3	8	3	4	3	7	7
Malouf *et al.*[5]	2000	18	7	2	2	12	n.a.	12	8	4	12
O'Brien and Skinner[6]	2000	13	2	2	0	3	1	1	4	3	5
Vaizey *et al.*[7]	1998	6	1	1	0	1	0	1	2	1	1
Dodi *et al.*[8]	2000	8	2	1	0	2	0	2	2	0	2
Present series	2002	22	3	5	2	9	2	7	2	10	9

*Involving anal cuff, connecting tubes, pump; †cuff leak, balloon rupture, pump leak, painful implant site. ‡Surgical reoperation in the postoperative period; §reoperation during follow-up. n.a., Not assessed

Table 3 Functional outcome in published literature

Reference	Year	No. of patients	Follow-up (months)*	Continence grading scale	
				Before implantation*	After implantation*
Wong *et al.*[3]‡	1996	7	40 (20–58)	n.a.	n.a.
Lehur *et al.*[4]§	2000	20	20 (6–35)	106(13)†	25(25)†
O'Brien and Skinner[6]‡	2000	10	n.a.	19 (18–20)	3 (0–6)
Vaizey *et al.*[7]§	1998	5	9 (4–12)	96·2 (70–108)	19·4 (0–61)
Dodi *et al.*[8]‡	2000	6	10·5 (4–23)	18·7(1·6)†	2·1(2·6)†
Present series‡	2002	15	26 (7–48)	18 (14–20)	4 (0–14)

Values are *mean (range) or †mean(s.d.). ‡Cleveland Clinical Florida Scale; §American Medical Systems incontinence score. n.a., Not assessed

Table 4 Mean resting anal canal pressure in published literature

Reference	Year	No. of patients	Mean resting anal canal pressure	
			Before implantation	After implantation
Wong *et al.*[3]	1996	7	16 mmHg*	68 mmHg*
Lehur *et al.*[4]	2000	20	28(17) mmHg†	60(16) mmHg†
Vaizey *et al.*[7]	1998	5	60 (30–80) cmH_2O‡	110 (100–120) cmH_2O‡
Dodi *et al.*[8]	2000	6	30 mmHg*	45 mmHg*
Savoye *et al.*[10]	2000	12	51(26) cmH_2O†	108(22) cmH_2O†
Present series	2002	15	35 (8–87) mmHg‡	54 (34–70) mmHg‡

Values are *mean, †mean (s.d.) or ‡mean (range)

study. These included infection, rupture of the cuff and loss of function of the device, skin erosion with exteriorization of different components of the system and perineal pain. A comparison of these complications between various published series is shown in *Table 2*. The rate of complications in most of these series was similar. It should be noted that in the study by Malouf *et al.*[5] there was a high rate of infection that in all cases was caused by methicillin-resistant *Staphylococcus aureus*. According to these findings, the authors suggested the need to detect and institute treatment for this pathogen both in patients and in staff members before operation.

All series reported a high reoperation rate and a significant proportion of patients had explantation of the device. In two series[3,4] as well as the current study, operations for placement of a new device were performed.

However, excluding the series by Wong *et al.*[3], the number of patients in whom definitive explantation of the device was necessary was high.

In the current study, as in others, significant improvements in continence and anal canal pressures after implantation of the neosphincter were recorded (*Tables 3* and *4*). However, comparison with other reports is difficult because the number of patients was small and different continence grading scales were used. Improvements in anal canal pressure generated after implantation of the cuff are difficult to compare owing to different laboratory reference values. Continence to solid (formed) stool was achieved in most patients evaluated in the different series. However, in most of the studies there was a significant number of patients with incontinence to liquid stool and flatus, who needed to wear pads and use astringent medication. Implantation of an artificial anal sphincter should therefore be recommended only for patients with severe incontinence to solid stool.

One of the problems documented in all series was obstructed defaecation. The cause of this problem remains unclear, although it may have occurred because the cuff closure was too tight[4]. A short duration of the time of cuff opening before it refills from the reservoir has been also suggested[10]. For this reason the routine use of laxatives or microenemas has been recommended[4]. Nevertheless, in another series[3] half of the patients were treated with antidiarrhoeal medication to improve continence. It may be possible that the use of some medication should be planned according to the patient's symptoms before operation.

In summary, this study of 22 patients with a mean follow-up of 28 months indicated that the Acticon Neosphincter® represents an alternative treatment for faecal incontinence that allowed re-establishment of continence to solid stool, although complete continence was achieved in only a few patients. However, the frequency of complications both in the immediate postoperative period and at follow-up, and the number of reoperations required, was very high.

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Original article

Permanent sacral nerve stimulation for treatment of idiopathic constipation

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Background: Constipation can usually be managed using conservative therapies. A proportion of patients require more intensive treatment. Surgery provides variable results. This paper describes an alternative approach, in which the neural control of the bowel and pelvic floor is modified, using permanent sacral nerve stimulation.

Methods: Four women (aged 27–36 years), underwent temporary and then permanent stimulation. All had idiopathic constipation, resistant to maximal therapy, with symptoms for 8–32 years. Clinical evaluation, bowel diary, Wexner constipation score, symptom analogue score, quality of life questionnaire and anorectal physiology were completed.

Results: There was a marked improvement in all patients with temporary, and in three with permanent, stimulation. Median follow-up was 8 (range 1–11) months. Bowel frequency increased from 1–6 to 6–28 evacuations per 3 weeks. Improvement occurred, at longest-follow-up, in median (range) evacuation score (4 (0–4) *versus* 1 (0–4)), time with abdominal pain (98 (95–100) *versus* 12 (0–100) per cent), time with bloating (100 (95–100) *versus* 12 (5–100) per cent), Wexner score (21 (20–22) *versus* 9 (1–20)), analogue score (22 (16–32) *versus* 80 (20–98)) and quality of life. Maximum anal resting and squeeze pressures increased. Rectal sensation was altered. Transit time normalized in one patient.

Conclusion: Permanent sacral nerve stimulation can be used to treat patients with resistant idiopathic constipation.

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Introduction

Constipation affects between 3 and 15 per cent of the general population at any one time[1,2]. Patients may experience a combination of decreased bowel frequency, difficulty in evacuation, abdominal pain and bloating, and often have a significantly impaired quality of life. In clinical terms it can be usefully defined as two or fewer spontaneous bowel movements per week, or straining for at least a quarter of the time[3]. In physiological terms patients can be classified as either having a prolonged whole-gut transit time[4], a disorder of pelvic floor coordination leading to impaired rectal evacuation[5], or a combination of the two. Organic and drug-associated causes account for a minority of patients, with the majority having idiopathic functional constipation.

Conventional treatment for idiopathic constipation initially involves dietary modification and pharmacological agents. These treatments can be associated with side-effects and results are often unsatisfactory[6]. Behavioural therapy, including biofeedback techniques, is usually only available at specialist centres; it will improve some patients. However, there remains a significant proportion of patients in whom conservative treatment fails[7]. In these patients surgical treatment may be considered. Surgical options for constipation involve either bowel resection or the formation of a stoma. Subtotal colectomy and ileorectal anastomosis is the most widely accepted surgical procedure. However, this has a significant associated morbidity with one-third of patients developing diarrhoea, 10 per cent remaining constipated and 10 per cent eventually progressing to a permanent ileostomy in the long term[8]. While a colostomy may relieve some of the symptoms of constipation it is not an attractive option for most patients, and symptoms of abdominal pain and bloating may remain[9].

Because of these limitations of conventional surgical treatment, an alternative surgical approach associated with less morbidity and reversibility would offer advantages. Modulating the neural control of bowel motility and pelvic floor function might offer such an approach for this functional condition. The concept of stimulating the nerve supply to the large bowel, to produce a physiological effect, was first applied therapeutically by Brindley[10], who inserted anterior sacral nerve root electrodes in patients with chronic spinal injury and associated bladder and bowel dysfunction. This treatment requires short stimulation at a high voltage. Patients with normal sensation would not tolerate such high voltages. After permanent implantation of anterior sacral nerve stimulators in patients with chronic spinal cord injury, high-pressure peristaltic waves could be induced electrically, and were associated with bowel evacuation[11,12].

In contrast to this approach, it may be possible to modify the neural activity of the innervation to the bowel and pelvic floor by applying a much lower-amplitude chronic stimulation, applied via electrodes through the sacral foramina. This technique, known as sacral nerve stimulation, has been applied successfully to the treatment of detrusor irritability and urinary retention[13,14], and most recently faecal incontinence[15–18].

In patients treated for urological disorders with chronic sacral nerve stimulation, a concomitant effect on bowel function has been noted. In a series of 250 permanent sacral nerve stimulator implants for bladder dysfunction 48 patients had coexisting symptoms of constipation. Retrospectively collected data from 36 of these patients showed that 78 per cent experienced a coincidental increase in bowel frequency and subjective improvement in defaecation (M. Stosser, unpublished data). Matzel *et al.*[18] reported the first use of sacral nerve stimulation to alter bowel motility in three patients treated successfully for resistant faecal incontinence. Several further studies have confirmed the beneficial clinical effects of both temporary and permanent sacral nerve stimulation in patients with faecal incontinence resistant to maximal conventional therapy[15,17,19,20].

Although this technique has been successful in both urinary retention and incontinence and resistant faecal incontinence, there has been little evaluation of its use primarily for constipation. Two studies have reported the effects of temporary stimulation. The first involved temporary stimulation for 3 weeks via a percutaneous electrode, and demonstrated a symptomatic improvement in two of eight patients with slow-transit constipation[21]. Transit time did not normalize in any of these patients. In a second report ten patients complaining of difficulty with rectal evacuation underwent temporary stimulation for 7 days[22]. Bowel frequency was reduced with stimulation, but difficulty with rectal evacuation, time for evacuation and number of unsuccessful attempts at defaecation all decreased. The number of patients who felt improved was not clear.

This report is the first to explore the value of this treatment with permanent stimulation. Four patients with chronic idiopathic constipation, resistant to maximal conventional therapy, underwent temporary and then permanent sacral nerve stimulation.

Patients and methods

Patients with severe idiopathic constipation were considered for treatment if they had failed to respond to maximal conventional therapy including laxatives and biofeedback therapy. Eligible patients had to have a bowel frequency of two or less per week and straining for more than a quarter of the time, for a minimum of 1 year. Exclusion criteria included previous abdominal surgery, hysterectomy, current or planned future pregnancy, and significant psychological disturbance (as judged clinically by the investigators).

Before any intervention the patients underwent thorough clinical investigation, including colonoscopy and proctography if required, to exclude other correctable causes of constipation, and a transit study. Whole-gut transit time was measured by a validated method involving the ingestion of three sets of radio-opaque markers on consecutive days and plain abdominal radiography at 120 h after ingestion of the first set of markers[23].

Suitable patients underwent temporary implantation for 3 weeks. Patients whose pretreatment bowel frequency at least doubled, who experienced a subjective improvement and had no complications during temporary stimulation were offered a permanent implant.

During all assessment (diary) periods, that is before treatment, during and after temporary stimulation, and during permanent implantation, patients were allowed only a 'rescue' laxative of 15 mg oral bisacodyl not more than each third day. This was taken only if needed, and recorded in the diary.

A bowel diary was completed, Wexner constipation and symptom analogue scores were measured, and quality of life assessment and anorectal physiological testing were undertaken before stimulation, at the end of 3 weeks of temporary stimulation, 1 month after temporary stimulation, before permanent implantation, and at 1, 3 and 6 months after permanent implantation.

A 3-week bowel diary documenting bowel frequency, difficulty with rectal evacuation, the presence and degree of abdominal pain and bloating, and laxative use was completed. Difficulty in evacuation was scored from 0, indicating no difficulty, to 4, indicating severe difficulty. The severity of constipation was assessed using the Wexner constipation score[24]. This questionnaire assesses different aspects of bowel function and generates a score from 0 (no symptoms) to 30 (worst symptoms). Patients were also asked to assess the overall severity of their symptoms on a visual analogue scale (best score 100, worst 0).

The effect of treatment on quality of life was assessed using the Short Form (SF) 36 health status survey[25]. This questionnaire consists of eight domains that assess impact of disease on physical and mental well-being.

Anal manometry was performed using a stationary pull-through technique with an eight-channel water-perfused system (MMS, Enschede, The Netherlands), which recorded maximal anal resting pressure and maximal squeeze pressure as the increment above resting pressure[26]. Rectal sensation to balloon distension with air at threshold, urge and maximum tolerated volume were recorded. Anal and rectal electrosensation to standard stimuli (5Hz, 0·1 ms and 10 Hz, 0·5 ms respectively) were recorded using a 1-cm bipolar ring electrode mounted on a 10-Fr catheter (Dantec 21L10, Neuromatic 2000 M/C current stimulator; Dantec Dynamics, Bristol, UK)[27].

The Harrow research ethics committee approved the study and all patients gave informed consent. Owing to the small number of patients results are presented in full, and were not analysed statistically.

Operative technique

The surgical procedure for temporary and permanent sacral nerve stimulation has been described previously[19,28]. Temporary electrodes were placed under general anaesthesia as a day-case procedure. Initially a 20-G, 89-mm, insulated spinal needle was inserted bilaterally into the S2, S3 and S4 foramina. A portable stimulator (Medtronic 3625; Medtronic, Minneapolis, Minnesota, USA) was used to elicit the best physical response of the pelvic floor and great toe to the lowest stimulus, judged both visually and electromyographically as the strongest contraction, indicating the optimal foramen for temporary stimulation. A helical percutaneous wire (Medtronic 3057) was inserted through the needle, connected to the portable stimulator and fixed carefully to the skin. Screening was performed for a 3-week period after which the wire was removed without analgesia as an outpatient procedure.

Permanent electrodes (Medtronic 3080) were placed surgically and secured into the same foramen as the previous temporary electrode under general anaesthesia. The impulse pulse generator (Medtronic 3023) was placed in the ipsilateral buttock, rather than the anterior abdominal wall, as earlier patients treated for incontinence had experienced pain related to the connecting wires over the iliac crest. This also decreased the operative time, as there was no requirement to turn and redrape the patient. All procedures were performed as a one-stage operation with prophylactic antibiotic cover.

Results

Four women, aged 27, 35, 36 and 36 years, underwent successful temporary percutaneous sacral nerve stimulation over a 3-week screening period, and subsequent permanent implantation. Two of these patients were included in a previous study of eight patients who underwent temporary stimulation[21]; these two patients showed a good clinical response to temporary stimulation and were therefore included in this study of permanent stimulation. The preoperative duration of symptoms was 8, 20, 28 and 32 years, and all patients had failed maximal treatment. All were on regular varied laxatives with a bowel frequency, on laxatives, of two or less per week. Whole-gut transit time was prolonged in two patients (patients 1 and 2) and normal in the other two (patients 3 and 4). A proctogram performed in the latter two patients showed delayed evacuation only.

Symptoms

Temporary 3-week screening

Screening was performed for 3 weeks (range 21–22 days) and was beneficial in all patients. There were no complications or lead dislodgements. During screening the bowel frequency increased from 1, 6, 1 and 5 evacuations per 3-week period to 9, 13, 13 and 10 respectively (*Fig. 1*). The score for ease or difficulty in evacuation decreased from 0, 4, 4 and 4 before to 0, 1, 1 and 3 with temporary stimulation. There was an improvement in the percentage time with abdominal pain and bloating (*Fig. 2*), the Wexner constipation score (*Fig. 3*) and the patient symptom analogue score (*Fig. 4*). During screening no patient required laxatives. Temporary screening wires were removed after 21 days without complication.

Patient evaluation was continued for the 3-week period after screening following removal of the temporary stimulation wire. Percentage time with abdominal pain and bloating and symptom analogue scores all returned to baseline values within 2–3 days after cessation of stimulation (*Figs 2* and *4*). Bowel frequency and laxative use also returned to baseline levels over the 3-week period.

Permanent implantation

The median time between temporary screening and permanent implantation was 9 (range 1–16) months. All patients underwent permanent implantation without

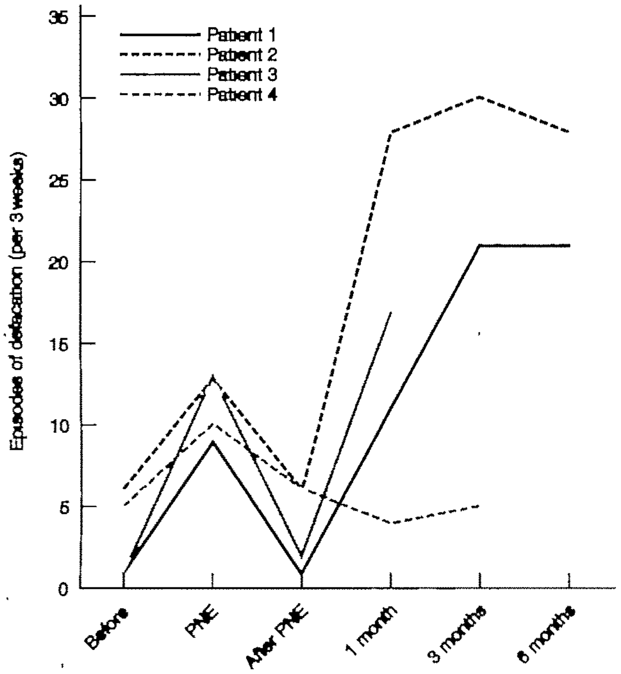

Fig. 1 Bowel frequency in four patients before sacral nerve stimulation; on the last day of a 3-week period of temporary stimulation (percutaneous nerve evaluation, PNE); after temporary screening, with no stimulation, before permanent implantation, and 1, 3 and 6 months after permanent implantation

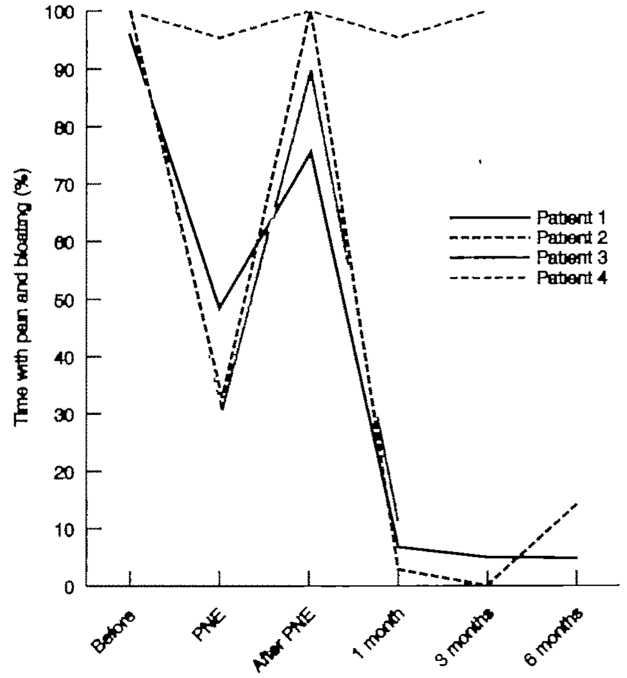

Fig. 2 Percentage time with abdominal pain and bloating (percentage of each 3-week period) in four patients before sacral nerve stimulation; on the last day of a 3-week period of temporary stimulation (percutaneous nerve evaluation, PNE); after temporary screening, with no stimulation, before permanent implantation; and 1, 3 and 6 months after permanent implantation

complication. The median operative time was 70 (range 60–100) min and patients were discharged at a median of 3 (range 2–4) days after operation.

The clinical effects of temporary sacral nerve stimulation were reproduced with permanent stimulation in three of the four patients, at a median follow-up of 8 (range 1–11) months (*Figs 1–4*). There was no deterioration in clinical benefit over time. Patient 4 underwent permanent implantation without complication with initial benefit, but was involved in a major road traffic accident 1 week after surgery. This caused movement of the implanted electrode and a return to baseline levels. This patient is currently being treated with adjustment of the electrode settings that allows for some flexibility, but may require surgical replacement of the lead.

All patients used regular laxatives before stimulation. None of the three patients with symptomatic improvement required laxatives during permanent stimulation.

Quality of life assessment was performed with the SF-36 health survey questionnaire. There was an improvement in all subscales, except health-transition, with both temporary and permanent stimulation.

Anorectal physiological and transit studies

Anorectal physiological testing showed a number of changes with both temporary and permanent stimulation. Anal manometry demonstrated a possible increase in maximal anal resting pressure, from median (range) 75 (52–99) cmH_2O before stimulation to 91 (72–114) cmH_2O with permanent stimulation, and maximal incremental squeeze pressure from 42 (32–102) to 63 (40–119) cmH_2O. Rectal sensation to distension improved, such that smaller volumes were required to elicit threshold sensation (median (range) 59 (45–71) *versus* 38 (30–45) ml air) and urge sensation (115 (90–185) *versus* 85 (50–90) ml air), and maximum tolerated volume decreased (157 (130–245) *versus* 125 (63–130) ml air). Rectal and anal sensation to electrical stimulation also improved; lower amplitudes were required to elicit anal threshold electrosensation (median (range) 6·4 (4·6–8·6) mA before *versus* 2·9 (2·6–6·0) mA with permanent stimulation) and rectal threshold electrosensation (29 (16–32) *versus* 14 (12–30) mA).

At baseline, before temporary stimulation, transit time was normal in two patients and slow in two. Transit time was remeasured in the latter two patients. With permanent

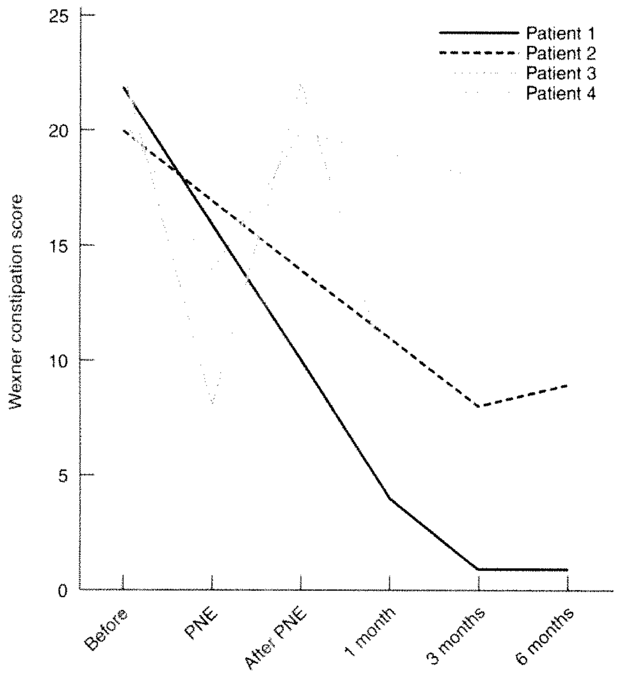

Fig. 3 Wexner constipation score (best, 0; worst, 30) in four patients before sacral nerve stimulation; on the last day of a 3-week period of temporary stimulation (percutaneous nerve evaluation, PNE); after temporary screening, with no stimulation, before permanent implantation; and 1, 3 and 6 months after permanent implantation. A Wexner score was not obtained on patients 1 and 2 during PNE

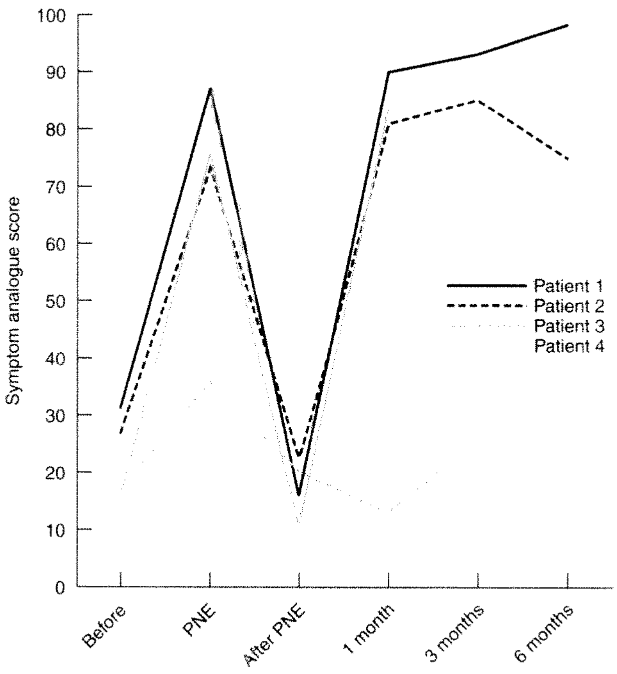

Fig. 4 Patient symptom analogue score (best, 100; worst, 0) in four patients before sacral nerve stimulation; on the last day of a 3-week period of temporary stimulation (percutaneous nerve evaluation, PNE); after temporary screening, with no stimulation, before permanent implantation; and 1, 3 and 6 months after permanent implantation

stimulation the transit time normalized in one patient (30 of 60 retained markers before compared with none after stimulation) but remained slow in the other patient (37 *versus* 50).

Stimulation parameters

Permanent stimulation was applied unilaterally to the S3 foramen in two patients and the S2 foramen in two patients. The foramen that had given the maximum acute response during electrode insertion, and which subsequently gave a good symptomatic response over the 3-week screening period, was selected for permanent stimulation.

Stimulation parameters were set at frequency of 14 Hz, pulse width 210 μs and amplitude to the threshold level of sensation, for 3-week screening and chronic stimulation. The median (range) stimulation amplitude was 1·7 (0·4–2·0) V. Stimulation was continuous with no deactivation for defaecation.

To assess whether different stimulation parameters may influence bowel function, four different stimulation frequencies were used in the first two patients with a permanent implant (5, 10, 25 and 50 Hz), each for a 3-week period after permanent implantation. No difference was observed in any of the parameters measured and the clinical outcome was not affected. Therefore chronic stimulation was set as for temporary screening.

Discussion

This study has demonstrated that permanent sacral nerve stimulation results in symptomatic improvement in patients with severe constipation. All patients had severe symptoms, had failed maximal conservative therapy and were considering the formation of a colostomy. With both temporary and permanent stimulation, patients experienced an increased bowel frequency, improved ease of evacuation, and improved associated symptoms of abdominal pain and bloating. Quality of life was also improved.

The beneficial clinical effect of temporary stimulation was reproduced in three patients with permanent stimulation. The fourth patient benefited with temporary, and initially with permanent, stimulation, but most likely dislodged the permanent electrode owing to trauma. A placebo response cannot be excluded as the cause of these three patients' symptomatic improvement. The return to pretreatment symptoms of constipation in the period between temporary and permanent stimulation, however, suggests that stimulation is responsible for symptom improvement. The authors have previously undertaken a double-blind assessment of the value of subthreshold stimulation and placebo in two patients with permanent sacral nerve stimulation for faecal incontinence[29], in which there was a clear response to active stimulation only. A similar study would be helpful in these patients with constipation.

This study involved a small group of patients. The authors believe that this kind of treatment should be not be widely applied to patients with constipation but should be restricted to patients who have failed other treatments, have major symptoms and are psychologically suited to intervention. This represents only a very small proportion of all patients.

Temporary stimulation is a minimally invasive procedure, of low morbidity, that was performed as a day-case procedure, but can be performed under local anaesthesia. It appears to provide a method of determining whether sacral nerve stimulation will be effective, before surgical intervention. However, a larger number of patients needs to be assessed and treated to determine its true positive predictive value. On removal of the temporary stimulation all clinical variables returned rapidly to baseline levels.

Implantation of a permanent sacral nerve stimulator is a relatively minor procedure, particularly compared with other surgical procedures for constipation, such as colectomy. The main risk is of infection of the implant; this risk is minimized by the use of prophylactic antibiotics, a one-stage technique with all material being implanted at the same operation, and the operative site being remote from the bowel. There were no operative complications in this series.

The stimulation parameters were set to the same levels used for faecal incontinence, with the amplitude determined by patient sensation. Altering the frequency did not appear to affect the clinical outcome; however, this was only tested in two patients for a 3-week period at each frequency. Further studies on the effect of different stimulation parameters are warranted.

Studies in patients with faecal incontinence have suggested that chronic stimulation has an effect on internal and external anal sphincter function, as well as anal and rectal motility[15,16,19]. Similar findings were seen in this study; there was an improvement in resting and squeeze pressures, and increased rectal sensitivity to distension and electrical stimulation. This suggests that stimulation had an effect on both afferent and efferent nerve fibres in these patients. Whether these physiological effects, or the clinical benefit seen in patients with idiopathic constipation occur via the same mechanism as that in patients with faecal incontinence is not known.

The long-term effect of chronic low-amplitude stimulation on the structures being innervated is unclear. Although animal studies have shown that chronic sacral nerve stimulation can alter the proportion of type I and type II muscle fibres in pelvic floor striated muscles, the effect on visceral structures is unknown. The mechanism by which sacral nerve stimulation modifies rectal and anal function in patients with idiopathic constipation also remains to be elucidated.

In this study patients both with slow transit, and normal transit with impaired evacuation, were helped. The number of patients with slow transit was too small in this study to evaluate the effect on transit. In contrast to the study of temporary stimulation that showed no improvement in bowel frequency[22], in the present study the three improved patients experienced an increase in bowel frequency and no longer required laxatives. Further studies are required to determine whether patients with predominately slow-transit constipation or those with mainly an impaired ability to evacuate will benefit most from stimulation.

The literature contains many references to a variety of anatomical abnormalities that have been associated with symptoms of constipation, including non-relaxing puborectalis, internal prolapse or enterocele. The authors regard these findings as subjective; they overlap with slow transit, and have varying incidence depending on the measurement techniques used. Patients were therefore not assessed for these factors as part of the present study.

The total cost of the equipment required for temporary and permanent sacral nerve stimulation is approximately £7000. The permanent stimulator accounts for the majority of this. In contrast, the total cost of subtotal colectomy is at least £5000. However, the operation is associated with a success rate of approximately 50 per cent and there is considerable morbidity[8]. In patients with permanent complete spinal cord injury a permanently implanted nerve stimulator is cheaper than long-term bowel care, with the operative technique coming into superior financial advantage at about 5 years[30].

Permanent sacral nerve stimulation appears to be a beneficial treatment for patients with severe symptoms of intractable constipation that has not responded to conservative treatments. As well as improved bowel frequency and ease of defaecation, symptoms of abdominal pain and bloating were improved. This study, reporting the initial results in a small group of patients, has shown that this technique may have future potential in an area in which current treatment is often unsatisfactory. Larger long-term studies are now required.

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Original article

Retrospective study of acute toxicity following short-course preoperative radiotherapy

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Background: The use of short-course preoperative radiotherapy (25 Gy in five fractions over 1 week) in resectable rectal cancer reduces local recurrence but is associated with an increased risk of postoperative complications and late toxicity. This study aimed to identify those patients who are unlikely to benefit from short-course preoperative radiotherapy and the factors associated with acute toxicity.

Methods: All patients who received short-course preoperative radiotherapy at a university hospital in 1998 and 1999 were included in this retrospective study. The association between complications occurring within 3 months and patient demographics, radiotherapy technique, surgical details and overall treatment time (OTT) was assessed by univariate and multivariate analysis.

Results: The mortality rate at 30 days was 6 per cent in the 177 patients identified. Thirty-seven per cent of patients had either Dukes' A tumours, surgically incurable disease or positive circumferential margins. One or more complications occurred in 38 per cent of patients. On multivariate analysis an OTT of more than 13 days ($P = 0.03$), age ($P = 0.02$) and length of the radiotherapy field ($P = 0.05$) were associated with an increased risk of complications.

Conclusion: Surgery within 1 week of completing short-course preoperative radiotherapy improved preoperative staging and use of an optimal radiotherapy technique will result in fewer patients at risk of acute toxicity.

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Introduction

Short-course preoperative radiotherapy (25 Gy in five fractions delivered over 5–7 days) has been shown to improve local control and increase overall survival in resectable rectal cancer[1–3]. The Swedish Rectal Cancer Trial showed a reduction in the 5-year local recurrence rate from 27 to 11 per cent and an increase in overall 5-year survival from 48 to 58 per cent when compared with surgery alone[1]. A recent literature-based meta-analysis of randomized trials that compared preoperative radiotherapy (between 5 and 45 Gy in 1–25 fractions) with surgery alone in patients with resectable disease has also shown a statistically significant survival advantage favouring the addition of preoperative radiotherapy to surgery, with an odds ratio (OR) of death at 5 years of 0·84 (95 per cent confidence interval (c.i.) 0·72 to 0·98) ($P = 0.03$)[4].

However, the exact role of short-course preoperative radiotherapy remains controversial for several reasons. The widespread introduction of subspecialization in colorectal surgery together with total mesorectal excision (TME) has resulted in lower rates of local recurrence than were observed in the surgery-alone arm of the Swedish Rectal Cancer Trial[5–7]. In the Stockholm I and II trials, patients had lower rates of local recurrence if operated on by surgeons who had been certified specialists for 10 years[8]. However, preliminary results of the Dutch TME trial suggest that, despite optimal surgery, the addition of short-course preoperative radiotherapy further reduces local recurrence. The local recurrence rate was 2 per cent at 2 years compared with 8 per cent in patients treated by surgery alone[9].

The benefit of short-course preoperative radiotherapy in patients with Dukes' A disease is uncertain. Although a detectable reduction in local recurrence was reported in patients with Dukes' A tumours in the Swedish Rectal Cancer Trial, there were more patients with node-negative tumours in the irradiated group (68 *versus* 59 per cent;

$P < 0.01$)[1]. Based on data from this trial and the Uppsala trial, a downstaging effect following short-course preoperative radiotherapy has been reported when the time between the first day of radiation and surgery, otherwise known as overall treatment time (OTT), is greater than 10 days[10]. This may have implications for the recommendation of adjuvant treatment. In the Dutch TME trial, no such effect was seen as the majority of patients (90 per cent) had an OTT of 10 days or less[11].

Patients found to have metastatic disease at operation are unlikely to benefit from short-course preoperative radiotherapy. Similarly, the risk of local recurrence in patients who have involved circumferential margins following short-course preoperative radiotherapy and surgery is not reduced, and they may benefit from a prolonged preoperative fractionation. Further radiotherapy given after operation is not recommended as the risk of permanent morbidity is extremely high: Svoboda *et al.*[12] reported an incidence of late complications of 84 per cent in patients who received a combined preoperative (20 Gy in four fractions over 2–3 days) and postoperative (20–40 Gy in 2-Gy fractions) treatment compared with 17 per cent in patients receiving the preoperative regimen alone.

Central to all these controversies is the potentially increased rate of acute and late toxicity seen with short-course preoperative radiotherapy. In the Stockholm I trial of short-course preoperative radiotherapy and the Rectal Cancer Group/Imperial Cancer Research Fund Trial, which used 15 Gy in three fractions over 5 days, the postoperative mortality rate was increased in the irradiated group[13,14]. This was largely due to infective and cardiovascular complications in patients irradiated to a large volume using a two-field technique[2,13–15]. In the Swedish Rectal Cancer Trial, in which patients were irradiated using three or four fields with an upper border at the L4–L5 vertebral level, compared with L1–L2 in the Stockholm I trial, the postoperative mortality rate was not increased but there was a greater incidence of perineal wound infection[16]. Such an increase has also been described in other trials of short-course preoperative radiotherapy[2,15,17] as well as in trials that used alternative preoperative fractionations[14,18–20].

An increase in late complications has also been reported following short-course preoperative radiotherapy. An excess of thromboembolism, femoral neck and pelvic fractures, intestinal obstruction and postoperative fistula in the irradiated group was reported in the Stockholm I and Stockholm II trials[21]. The technique used in the former trial was, as noted above, suboptimal and included irradiation of the inguinal nodes thereby including the head and neck of both femurs. Despite the use of modern radiotherapy techniques in the Swedish Rectal Cancer Trial, patients reported an increase in faecal incontinence and urgency[22], which may be explained by irradiation of the anal canal.

A planned volume technique using a three- or four-field beam arrangement is the current recommendation based on experience from previous trials. The superior border of the volume should be at the level of the sacral promontory and the anal canal can be excluded for tumours more than 6 cm above the anal margin[23]. The lateral margins of the volume are placed 0·5–1·0 cm outside the pelvic side-wall thus sparing the femora from the high-dose volume. Shielding of the sacral plexus from the lateral beams may reduce the incidence of acute sacral plexopathy[23]. Despite these modifications, there is still a potential for increased early and late toxicity, and it is therefore appropriate to avoid short-course preoperative radiotherapy in patients who are unlikely to benefit.

The aim of this retrospective study was twofold: first, to identify the proportion of patients who were unlikely to benefit from short-course preoperative radiotherapy (Dukes' A tumours, metastatic or unresectable disease, positive circumferential margin) and, second, to identify factors that may be related to acute or postoperative morbidity.

Patients and methods

Data collection

All patients with resectable rectal adenocarcinoma referred for short-course preoperative radiotherapy to the Queen Elizabeth Hospital, Birmingham between 1 January 1998 and 31 December 1999 were identified. Hospital notes, operation notes, histopathological reports and radiotherapy plans were examined to obtain the following information: age, sex, referring hospital, radiotherapy technique (beam energy, dimensions of posterior field, beam arrangement), surgical details (OTT, operation type, duration of admission), pathological details (Dukes' stage, circumferential margin) and mortality and morbidity within 3 months of short-course preoperative radiotherapy.

Radiotherapy

All patients were simulated before treatment. In a minority of cases two parallel opposing fields were used to cover the treatment volume defined by the supervising radiotherapist. In the majority of cases three fields (one posterior and two posterior oblique or two lateral fields) were used. A radiation dose of 25 Gy in five daily fractions was prescribed to the intersection point over 5–7 days using megavoltage photons (5 or 8 MV).

Surgery

Surgery was performed at one of eight referring units. The intended operation included TME in all patients.

Morbidity

For the purpose of this analysis complications were defined as those occurring within 3 months of the first day of radiotherapy. They were classified according to the diagnosis recorded in the clinical notes: wound (perineal or abdominal wound, dehiscence or infection requiring antibiotic treatment), pelvic sepsis (documented clinical evidence of sepsis from a pelvic source), anastomotic leak (with radiological confirmation), cardiovascular (myocardial infarction, unstable angina, stroke), thromboembolic (deep vein thrombosis, pulmonary embolus), haemorrhage (requiring pelvic packing and/or reoperation), pneumonia, tumour perforation, obstruction, fistula or not known (sudden death cause unknown).

Statistical analysis

Patients were categorized into two groups: those who experienced complications within 3 months of commencing short-course preoperative radiotherapy and those who did not. These two groups were compared in terms of demographics, radiotherapy technique and surgical details. The χ^2 test was used to compare the categorical variables sex, radiotherapy beam energy, radiotherapy technique, operation performed, circumferential margin status, Dukes' stage and OTT (categorized as less than 10 days *versus* 10 days or more, less than 13 days *versus* 13 days or more, less than 20 days *versus* 20 days or more). The categories of OTT were based on the timing of surgery seen in the Dutch TME trial[9], the Swedish Rectal Cancer Trial[16] and the Lyon R90-01 trial[24]. The Wilcoxon test was used for age, field length, field width, length of inpatient stay and OTT as a continuous variable.

The combined effect of all the above variables was analysed by logistic regression; this included consideration of OTT as a continuous variable and in terms of the three categorizations. The regression analyses used a stepwise selection method to identify the most significant explanatory variables with an entry criteria of $P = 0{\cdot}05$ and a staying criteria of $P = 0{\cdot}05$.

All P values are two sided. $P < 0{\cdot}05$ was considered statistically significant.

Results

One hundred and seventy-seven patients were referred from eight units during this interval. Demographic and radiotherapy details were available in 176 patients (99 per cent), and surgical and pathological details in 175 patients (99 per cent).

Of these 175 patients, 166 (95 per cent) underwent resection. Twenty-nine patients (17 per cent) were found to have Dukes' A, 72 (41 per cent) Dukes' B and 65 (37 per cent) Dukes' C disease. The remaining nine patients (5 per cent) were explored but not resected owing to locally advanced or metastatic disease. A curative resection was performed in 159 patients (91 per cent): 140 (80 per cent) had negative circumferential margins and 19 (11 per cent) had involved margins. In addition, seven patients (4 per cent) had a palliative resection (*Table 1*).

Within 3 months of commencing radiotherapy, 67 patients (38 per cent) developed one or more complications (*Table 2*) including 15 deaths (9 per cent). Ten of these deaths (6 per cent) were within 30 days of surgery. Age ($P = 0{\cdot}01$), radiotherapy field length ($P = 0{\cdot}05$) and OTT (expressed as a continuous variable ($P = 0{\cdot}01$), or

Table 1 Surgical outcomes

	Dukes' stage				
Resection type	A	B	C	No resection	Total
R0	29	63	48	–	140 (80)
R1	–	7	12	–	19 (11)
R2/metastasis	–	2	5	9	16 (9)
Total	29 (17)	72 (41)	65 (37)	9 (5)	175*

Values in parentheses are percentages. *In two patients both Dukes' stage and resection type are missing

Table 2 Complications occurring within 3 months of commencement of short-course preoperative radiotherapy

	No. of cases
Wound	23 (13)
Pelvic sepsis	11 (6)
Anastomosis	10 (6)
Cardiovascular	6 (3)
Thromboembolic	6 (3)
Haemorrhage	5 (3)
Pneumonia	6 (3)
Tumour perforation	3 (2)
Obstruction	3 (2)
Fistula	1 (1)
Not known	1 (1)

Values in parentheses are percentages. Of 176 patients in whom information was available, 67 (38 per cent) experienced 75 complications; 59 (34 per cent) experienced one complication and eight (5 per cent) experienced two complications as categories less than 10 days *versus* 10 days or more (P = 0·03) or less than 13 days *versus* 13 days or more (P = 0·04)) were significantly associated with complications (*Table 3*).

Logistic regression analysis (*Table 4*) selected age, OTT (expressed as less than 13 days *versus* 13 days or more) and radiotherapy field length as significant explanatory variables for the probability of complications. If OTT was considered as a continuous variable, it remained in the logistic regression model as a significant factor.

OTT less than 13 days was associated with a decreased risk of complications (P = 0·03); the risk for an OTT of less

Table 3 Univariate analysis of factors potentially associated with complications

	With complications	Without complications	P‡
No. of patients*	67 (38)	109 (62)	
Age (years)†	70 (65–77, 32–95)	67 (56–75, 22–84)	0·01
Sex*			0·55
Male	46 (40)	70 (60)	
Female	21 (35)	39 (65)	
Energy (MV)*			0·79
5	16 (36)	28 (64)	
8	51 (39)	81 (61)	
Technique*			0·86
Three field	59 (38)	95 (62)	
Two field	8 (36)	14 (64)	
Field length (cm)†	17·2 (16·4–18·7, 13·0–22·0)	17·0 (15·6–18·2, 9·4–24·3)	0·05
Field width (cm)†	15·0 (13·5–16·0, 11·5–18·0)	15·0 (13·0–15·5, 11·0–18·5)	0·48
Overall treatment time (days)			
As continuous variable†	14 (10–18, 7–28)	10·5 (9–14, 5–37)	0·01
Cut-off 10 days*			0·03
< 10	14 (26)	39 (74)	
⩾ 10	53 (43)	69 (57)	
Cut-off 13 days*			0·04
< 13	31 (32)	67 (68)	
⩾ 13	36 (47)	41 (53)	
Cut-off 20 days*			0·16
< 20	53 (36)	94 (64)	
⩾ 20	14 (50)	14 (50)	
Operation type*			0·41
Abdomino-perineal	28 (41)	40 (59)	
Anterior resection	34 (38)	56 (62)	
Other	4 (24)	13 (76)	
Surgical outcome*			0·92
R0	52 (37)	88 (63)	
R1	8 (42)	11 (58)	
R2/metastasis	6 (38)	10 (62)	
Dukes' stage*			0·93
A	12 (41)	17 (59)	
B	28 (39)	44 (61)	
C	23 (35)	42 (65)	
No resection	3 (33)	6 (67)	

*Values in parentheses are percentages; †values are median (interquartile range, range). Data on operation type, surgical outcome and Dukes' stage are missing for one patient with complications, and on overall treatment time for one patient without complications. ‡Wilcoxon test for continuous variables and χ^2 test for categorical variables

Table 4 Factors selected in logistic regression analysis

	Parameter estimate	Standard error	Test statistic	P	Odds ratio
Overall treatment time < 13 days	− 0·69	0·33	4·52	0·03	0·50 (0·26, 0·95)
Age	0·03	0·01	5·55	0·02	1·04 (1·01, 1·07)
Field length	0·15	0·08	3·93	0·05	1·16 (1·00, 1·35)

Values in parentheses are 95 per cent confidence intervals

than 13 days was half that of a longer OTT (OR 0·50 (95 per cent c.i. 0·26 to 0·95)). Increasing age ($P = 0.02$) was associated with complications, with the risk increasing by 4 per cent for every year of age. Increasing length of radiation field ($P = 0.05$) was also associated with risk of complications and this was independent of operation type.

Patients with complications remained in hospital significantly longer than those without complications (median (interquartile range) duration of hospital stay 18 (14–32) *versus* 11 (10–14) days; $P < 0.001$).

Discussion

Although preoperative radiotherapy appears to improve survival compared with surgery alone, this benefit is not apparent in patients with Dukes' A tumours[4]. In the Dutch TME trial, short-course preoperative radiotherapy had no effect on local recurrence in patients with stage I and IV disease[9]. Similarly, an update of the Stockholm II trial showed that only patients undergoing curative resection benefited from short-course preoperative radiotherapy[25]. The prognosis of those with metastatic or macroscopic residual disease was not altered[25]. If such patients were identified preoperatively, they could be spared the potential toxicity of short-course preoperative radiotherapy.

In this retrospective analysis, 29 patients (17 per cent) had Dukes' A tumours and 16 (9 per cent) were incurable because of unresectable tumours or metastatic disease. A further 19 resected patients (11 per cent) had positive circumferential margins. If more accurate staging were available, the management of 37 per cent of patients would have been different.

The accuracy of endorectal ultrasonography in identifying T1 and T2 tumours is between 75 and 85 per cent[26]. Similar results have been reported with endoscopic ultrasonography[27]. At the other extreme, the accuracy of computed tomography (CT) in detecting T4 tumours is 81 per cent[28]. In a review of preoperative staging, Heriot *et al.*[29] recommended endorectal ultrasonography for early tumours, magnetic resonance imaging (MRI) for locally advanced tumours and ultrasonography or CT for assessment of the liver. Use of MRI can predict the risk of circumferential margin involvement[30]. Therefore, with the routine preoperative use of endorectal or endoscopic ultrasonography and CT, and MRI in appropriate cases, the selection of patients for short-course preoperative radiotherapy could be improved.

The acute complication rate of 38 per cent in this study is comparable with that in the Swedish Rectal Cancer Trial[16] as well as audit data from the North Trent region[31]. Of the factors related to radiation technique, the irradiated field length was the only significant co-variate. A similar finding has been reported in a Welsh retrospective audit[32]. In the future, the use of conformal radiotherapy planning and delivery techniques may reduce the risk of toxicity.

Several trials have reported an association between increasing age and postoperative morbidity and mortality[2,14,33,34]. In the Stockholm I trial, the 30-day mortality rate of patients receiving radiotherapy was 16 per cent for patients aged over 75 years, 5 per cent in younger patients, and 2 per cent in patients in both age groups who had surgery alone. This is consistent with the present finding that age is associated with an increased risk of complications. Selection of elderly patients, particularly with respect to cardiovascular co-morbidity, is required.

In the Lyon R90-01 randomized trial, which examined the effect of delayed surgery following preoperative radiotherapy, there was no difference in acute morbidity between operating at 2 weeks or 6–8 weeks following 39 Gy in 13 fractions over 17 days[24]. However, these results may not be applicable to short-course preoperative radiotherapy. Surgery within 7 days of completing such radiotherapy is within the protocol for the ongoing Medical Research Council CR07 trial[35]. Only 12 per cent of patients in the Swedish Rectal Cancer Trial were operated on beyond this interval and no relationship between postoperative mortality and OTT was seen[16]. In the North Trent Audit there appeared to be an increased risk of perineal wound breakdown in patients operated on more than 7 days after short-course preoperative radiotherapy (30 *versus* 15 per cent) but this did not reach statistical significance ($P = 0.36$)[31]. In the present study, 44 per cent of patients were operated on beyond this interval and the risk of complications was significantly higher ($P = 0.03$). Similarly, in the Dutch TME trial, there was an increased postoperative mortality rate (4 *versus* 8 per cent; $P = 0.02$) in patients operated on after a delay of more than 3 days following completion of short-course preoperative radiotherapy[36].

In summary, more accurate preoperative staging and improvement in radiotherapy technique may result in fewer patients at risk of acute complications. Surgery within 1 week of completion of short-course preoperative radiotherapy reduces the risk of postoperative complications and avoids tumour downstaging.

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Original article

Medium-term results of permanent sacral nerve stimulation for faecal incontinence

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Background: Anal sphincter surgery for faecal incontinence is associated with significant morbidity and a variable outcome. Sacral nerve stimulation may provide a good functional outcome with minimal morbidity. This paper reports the experience in a single centre over 5 years.

Methods: Fifteen consecutive patients (14 women), median age 60 (range 37–71) years, underwent temporary, and subsequent permanent, stimulation. All had incontinence to solid or liquid stool; the aetiology was obstetric injury (seven patients), scleroderma (four), idiopathic (two), fistula surgery (one) and repaired rectal prolapse (one). Median duration of symptoms was 6 (range 2–15) years. Clinical evaluation, endoanal ultrasonography, bowel diary, quality of life questionnaire (Short Form (SF) 36) and anorectal physiological testing were performed before and after stimulation.

Results: Continence had improved in all patients at median follow-up of 24 (range 3–60) months. Eleven patients were fully continent. Episodes of faecal incontinence decreased from median (range) 11 (2–30) per week before stimulation to 0 (0–4) per week after permanent stimulation ($P < 0.001$). Urgency improved in all patients (median (range) ability to defer less than 1 (0–1) *versus* 8 (1–15) min; $P = 0.01$). 'Social function' and 'role-physical' subscales of the SF36 improved significantly. Mean resting pressure (35 *versus* 49 cmH_2O with temporary stimulation; $P < 0.05$) and squeeze pressure increment (43 *versus* 69 cmH_2O with permanent stimulation; $P < 0.01$) increased. Rectal sensitivity to initial distension changed (mean 47 *versus* 34 ml air; $P < 0.05$). There were no major complications.

Conclusion: Sacral nerve stimulation is a safe and effective treatment for faecal incontinence when conventional treatment has failed. There is minimal morbidity. The benefit is maintained in the medium term.

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Introduction

Faecal incontinence affects approximately 2 per cent of the adult general population[1]. Conservative management involves dietary advice, antidiarrhoeal medication and behavioural therapy (biofeedback)[2,3]. While these measures will improve many patients there remains a proportion with resistant symptoms[4].

Anal sphincter surgery has been the focus of the approach in patients who have failed conservative treatments and in whom symptoms are severe. When there is disruption to the external anal sphincter, related to previous obstetric or other trauma, an overlapping sphincter repair produces a good symptomatic result in 70–80 per cent of patients in the short term[5]. However, in the medium term the results of sphincter repair deteriorate, with no patient maintaining full continence and only 50 per cent having improved continence[6]. Other newer surgical approaches such as the dynamic graciloplasty and artificial bowel sphincter can improve continence, but both procedures involve major surgery, and have a high associated morbidity and substantial failure rate[7,8]. A permanent stoma is a further alternative.

Because of the invasive nature of sphincter surgery, and the poor outcome in many patients, recent attention has focused on non-invasive or minimally surgically invasive therapies. Topical pharmacological agents that raise sphincter pressure[9] or prevent sphincter relaxation may have a role in treating incontinence, but clinical efficacy has been established only in patients with an ileoanal reservoir[10].

An alternative approach involves modifying the nervous system that controls lower bowel, anal sphincter and pelvic floor function. Sacral nerve stimulation is a technique that uses low-level electrical stimulation, applied via electrodes through the sacral foramina, to the nerves of the sacral plexus. This approach of neural modulation to treat a functional condition has been used safely and successfully for a number of years to treat urinary disorders[11]. Following the observation that sacral nerve stimulation affected bowel function in patients treated for urinary disorders, it was then applied to patients with faecal incontinence. In the first report of such use this technique was used successfully to treat three patients with chronic faecal incontinence resistant to conventional therapy[12]. Further studies have shown similar successful results in the short term with both temporary and permanent stimulation in small numbers of patients[13–17]. This report highlights the results from one institution over 5 years in a consecutive series of patients with faecal incontinence treated with sacral nerve stimulation.

Patients and methods

Fifteen patients (14 women), median age 60 (range 37–71) years, underwent initial temporary stimulation, and then implantation with a permanent sacral nerve stimulator between October 1996 and May 2001. All patients had faecal incontinence to solid or liquid stool at least twice per week and had failed to improve with maximal conventional treatment, including antidiarrhoeal agents and behavioural therapy (biofeedback).

The median preoperative duration of symptoms was 6 (range 2–15) years. The aetiology of incontinence was obstetric injury ($n = 7$), systemic sclerosis ($n = 4$), idiopathic ($n = 2$), perianal fistula surgery ($n = 1$) and repaired recurrent complete external rectal prolapse ($n = 1$). Five patients had undergone previous anal sphincter repair but had persistent incontinence, despite either a partial or complete overlap of the external anal sphincter demonstrable on endoanal ultrasonography. No patient had a fully disrupted external anal sphincter but four patients had a disrupted or fragmented internal anal sphincter shown ultrasonographically. All patients had at least a demonstrable unilateral pudendal nerve terminal motor latency and in 13 patients it was normal bilaterally.

Before stimulation all patients had a full clinical assessment, completed a 3-week bowel habit diary and the Short Form (SF) 36 Health Survey quality of life assessment[18], and underwent endoanal ultrasonography[19] and anorectal physiological testing. The diary, quality of life assessment and anorectal physiological testing were repeated during the last day of temporary stimulation, with the temporary electrode still *in situ*, and at 3, 6 and 12 months and then annually after permanent implantation.

Physiological testing included anal manometry, rectal sensation to balloon distension at threshold, urge and maximal tolerated volume, anal and rectal electrosensory testing, and determination of pudendal nerve terminal motor latency. Manometry was performed using an eight-channel water-perfused system (MMS, Enschede, The Netherlands) by a stationary pull-through technique that assessed both maximum resting anal pressure and maximum squeeze pressure. The latter was recorded as the maximum voluntary increment above resting pressure[20]. Rectal sensation was tested to balloon distension with air. Anal and rectal sensation to electrical stimulation were tested using a 1-cm bipolar ring electrode mounted on a 10-Fr catheter. Stimulation parameters were 5 Hz, 0·1 ms and increasing amplitude for anal sensation, and 10 Hz, 0·5 ms and increasing amplitude for rectal sensation[21]. Pudendal nerve terminal motor latency was recorded with the St Mark's glove electrode[22].

Operative technique

The surgical technique and equipment for temporary and permanent sacral nerve stimulation have been described previously[23]. Modifications are described here. Preliminary temporary screening in a previous study[24] had employed a percutaneous electrode (Medtronic 004; Medtronic Interstim, Minneapolis, Minnesota, USA) with a portable temporary stimulator (Medtronic 3625). However, this had occasionally displaced from the sacral foramen during the temporary screening period. For this reason four of the earlier patients underwent temporary screening with a surgically implanted fixed sacral electrode (Medtronic 3080), connected to an external non-implanted battery stimulator. The subsequent 11 patients underwent temporary screening using a percutaneous needle and newly developed helical wire electrode (Medtronic 3057) which had less tendency to dislodge. In these 11 patients the temporary electrode was removed before one-stage permanent implantation.

Patients proceeded to permanent implantation if there was at least a 50 per cent improvement in continence, judged by episodes of faecal incontinence, and no serious complications. The first nine patients were implanted with a permanent impulse generator (Medtronic 3023) placed in the anterior abdominal wall, superficial to the rectus muscle. Three patients experienced pain over the connecting wires that had been tunnelled subcutaneously over the iliac crest. For this reason the impulse generator was placed below the superficial fascia in the buttock, ipsilateral to the permanent electrode, in the last six patients. This also decreased operative time, as there was no requirement to turn and redrape the patient during surgery.

Prophylactic intravenous cefuroxime, metronidazole and gentamicin were administered at the onset of surgery. Gentamicin solution was used to soak all implanted equipment. Cephalexin and metronidazole were administered orally for 3 days after operation.

The Harrow Research Ethics Committee granted ethical approval for this study and all patients gave informed consent. Statistical analysis was performed with the Wilcoxon paired rank sum test.

Results

Clinical data

Continence had improved in all patients at a median follow-up of 24 (range 3–60) months. In all patients the initial benefit was maintained at final follow-up (*Table 1*). Eleven of 15 patients were fully continent at longest follow-up. Episodes of faecal incontinence decreased from median (range) 11 (2–30) per week before stimulation to 0 (0–7) and 0 (0–4) per week after temporary and permanent stimulation respectively ($P < 0{\cdot}001$) (*Table 1*). The symptom of urgency improved in all patients: median (range) ability to defer defaecation less than 1 (0–1) min before stimulation *versus* 8 (1–15) min at time of longest follow-up ($P = 0{\cdot}01$).

Temporary screening was performed for a median of 21 (range 7–42) days. Permanent implantation was performed at a median of 1 (range 1–4) month after removal of the temporary electrode. All 15 patients responded to temporary stimulation, with no failures, and this was reproduced with permanent implantation (*Table 1*). The median time spent as an inpatient for permanent implantation was 4 (range 2–5) days.

Table 1 Clinical effect of sacral nerve stimulation

	Mean no. of episodes of faecal incontinence per week								
			Follow-up (months)						
Patient	Before stimulation	PNE	3	6	12	24	36	48	60
1	15	0	0	0	0	0	0	0	0
2	5	0	1	0	2	0	0	0	1
3	20	7	0	0	8	0	0	0	
4	2	0	0	0	0	0	0	0	
5	11	0	0	1	0	0	1		
6	23	2	4	4	7	0			
7	30	2	5	4	7	0			
8	3	0	0	0	0	0			
9	12	2	1	2	6	4			
10	12	1	2	3	0				
11	7	0	1	0					
12	29	2	2	2					
13	4	0	0	0					
14	7	0	0						
15	7	0	0						
Median	11	0*	0*	0*	2†	0†	0‡	0	0

PNE, percutaneous nerve evaluation. *$P < 0{\cdot}001$, †$P < 0{\cdot}01$, ‡$P < 0{\cdot}05$ *versus* baseline (Wilcoxon signed rank test)

There was an improvement in all subscales, except health transition, of the SF36 quality of life questionnaire (*Table 2*). This was statistically significant ($P < 0{\cdot}05$) in the role-physical and social function subscales.

Physiological testing

Anal manometry showed a significant increase in maximum anal resting pressure during temporary stimulation (mean(s.d.) 35(17) cmH$_2$O before stimulation *versus* 49(21) cmH$_2$O after temporary stimulation; $P < 0{\cdot}05$). Although this increase was maintained with permanent stimulation (41(19) cmH$_2$O) it was not statistically significant ($P = 0{\cdot}2$). There was a significant increase in maximal squeeze pressure increment during stimulation: mean(s.d.) 43(40) cmH$_2$O before stimulation *versus* 74(47) cmH$_2$O with temporary stimulation ($P < 0{\cdot}01$) and 69(49) cmH$_2$O with permanent stimulation ($P < 0{\cdot}05$).

The threshold of rectal sensation to balloon distension became more sensitive with permanent stimulation: mean(s.d.) 47(19) ml air before stimulation *versus* 34(15) ml air after permanent stimulation ($P < 0{\cdot}05$). Other physiological variables did not change significantly during temporary or permanent stimulation (*Table 3*).

Stimulation parameters

Based on the optimal response to stimulation during initial temporary electrode placement, stimulation was applied unilaterally to the S3 foramen in 14 patients and to the S4

Table 2 Short Form 36 quality of life assessment

Category	Before stimulation	Last follow-up*
Role-emotional	0(41)	33(49)
General health	37(27)	40(31)
Mental health	36(27)	60(26)
Bodily pain	50(26)	40(30)
Physical functioning	60(32)	75(38)
Role-physical	0(36)	75(49)†
Social function	37(31)	75(30)†
Vitality	25(24)	35(28)
Health transition	60(27)	60(24)

Values are median(s.d.). *Median 24 months. †$P < 0{\cdot}05$ *versus* baseline (Wilcoxon signed rank test) foramen in one patient. The same foramen was later used for permanent stimulation.

Temporary stimulation was supplied by a portable stimulator (Medtronic 3625) at frequency of 15 Hz, pulse width 210 μs, continuous mode and amplitude at the threshold of patient sensation. Permanent stimulation was supplied by the implanted pulse generator (Medtronic 3023) set with the same stimulation parameters. The median amplitude, usually just above sensory threshold, was 1·9 (range 0·1–5·0) V. Minor modifications to stimulation amplitude and polarity of the four stimulation points were necessary in some patients to achieve the optimal clinical effect. The stimulation was not turned off for defaecation.

Complications

There were no major complications or infections of permanent implants and no implants have had to be removed. One patient who was taking corticosteroids had a superficial skin infection during percutaneous screening that resolved after removal of the temporary electrode; permanent implantation proceeded 1 month later. Permanent lead dislodgement occurred in two patients, one at 3 days after operation and one at 1 year. Both were early in the series and the leads were replaced surgically with good result. Three patients experienced pain at the iliac crest over the subcutaneous connecting wires. This resolved with repeated local anaesthetic and steroid injections and did not occur in the later implants to the buttock.

Some patients occasionally experienced minor localized electric shocks when passing through ambient electrical or magnetic fields. Deactivation of the pulse generator magnet within the permanent stimulator, but not the stimulator itself, eliminated this problem.

Discussion

This study has demonstrated that sacral nerve stimulation results in marked symptomatic improvement in patients with severe faecal incontinence of varying aetiologies. All patients had severe symptoms, had failed maximal conventional therapy and were considering the formation of a colostomy. With both temporary and permanent stimulation all patients experienced a marked improvement in continence and an increased ability to defer defaecation. The majority were fully continent and this beneficial effect did not deteriorate with time.

There was a significant improvement in quality of life as measured by the SF36 Health Survey questionnaire. In some patients, however, this was not marked, probably because of persistent symptoms from associated disease. For example, the four patients with progressive systemic sclerosis had other symptoms; one of these patients required a limb amputation, after the stimulator had been implanted.

There are few data on the results of this therapy for faecal incontinence in the literature. Matzel and colleagues[12] published the first report in three patients who were followed up for 6 months. All three patients benefited from treatment. The authors' centre subsequently reported on five patients who had a median follow-up of 16 months, all of whom had a good symptomatic result[15]. The number of patients was too small to demonstrate a consistent change in physiological parameters. This centre also reported a double-blind cross-over study, with and without stimulation, in two patients[25]; a marked symptomatic benefit was shown and, although the number of patients was small, it was demonstrated that the benefit from this treatment is unlikely to be related to placebo. The prolonged clinical benefit seen in the present study, without deterioration, further supports the lack of a placebo effect.

Subsequent reports all involved short-term follow-up or a small number of patients. Matzel *et al.*[16] reported on six patients; the stimulation appeared to result in an increase in squeeze pressure but two patients had to have the stimulus generator removed because of pain. Leroi *et al.*[14] reported on five patients, three of whom showed clinical benefit at 6 months' follow-up. Ganio and colleagues[13] implanted five patients with a permanent stimulator; after a median of 19 months there was an increase in resting pressure and a

Table 3 Anorectal physiology results

	Before stimulation	PNE	Last follow-up*
Mean resting pressure (cmH_2O)	35(17)	49(21)‡	41(19)
Mean squeeze pressure increment (cmH_2O)	43(40)	74(47)†	69(49)‡
Mean threshold volume (ml air)	47(19)	65(33)	34(15)‡
Mean urge volume (ml air)	82(31)	106(48)	74(41)
Mean maximum tolerated volume (ml air)	127(43)	150(52)	103(49)

Values are mean(s.d.). *Median 24 months. PNE, percutaneous nerve evaluation (recorded at the end of 3-week screening period). †$P < 0·01$, ‡$P < 0·05$ *versus* baseline (Wilcoxon signed rank test) possible increase in squeeze pressure. The most recent and largest published report, from Rosen *et al.*[17], described 16 patients implanted initially, but four patients subsequently had the device removed owing to infection or displacement. At a median follow-up of 15 months patients had a marked improvement in incontinence, which appeared to be associated with an increase in resting and squeeze anal pressures.

The current report is the largest series to date, and includes patients with the longest follow-up. Although the median follow-up of 2 years is still relatively short for a surgical procedure, this study nevertheless provides important data about the medium-term efficacy and safety of this treatment.

In this study the initial patients were screened with a surgically placed electrode (Medtronic 3080) to avoid premature lead dislodgement. With the development of the helical percutaneous screening wire (Medtronic 3057), designed to resist dislodgement, there were no failures so all further screening was performed percutaneously. This technique does not involve open surgery, has a lower infection risk, a very low morbidity and can be performed using local anaesthetic. Both local and general anaesthesia were used for temporary electrode placement. Later patients usually had a general anaesthetic, for reasons of patient comfort and to allow time for optimal foramen selection.

The clinical effect of temporary stimulation was reliably reproduced in all patients with permanent stimulation. Percutaneous temporary stimulation therefore appears to provide a minimally invasive and accurate predictor of success before surgical intervention. This is a major advantage of the technique. In this series all patients had a successful temporary screening but larger series are required to be certain of the exact predictive value of the response to temporary stimulation.

Implantation of a permanent sacral nerve stimulator is a relatively minor procedure, especially compared with the alternative surgical options for these patients. The operative site is distant from the bowel so previous anal procedures in a patient do not make the surgery more complicated. The spinal site also has a lower infection risk. A one-stage surgical procedure performed after successful temporary screening further decreases the infection risk. Implantation of the stimulator into the ipsilateral buttock in the latter patients was without complications; it avoided iliac crest pain and shortened operative time. There were no major complications in this series and no implants had to be removed.

The finding of an increased squeeze pressure in this study adds weight to the body of evidence suggesting that this treatment does indeed augment striated anal sphincter muscle activity. The mechanism for this effect is unknown, but may involve hypertrophy of existing muscle fibres, alteration in muscle fibre type or the recruitment of redundant motor units in response to chronic stimulation. Further studies may help elucidate this. Given all the available evidence the effect on the resting anal pressure is less clear. These manometric changes are not necessarily the only, or even the relevant, mechanism by which this treatment improves continence. A qualitative change in rectal and anal sphincter smooth muscle function with temporary stimulation has also been shown previously[24].

The current study has also demonstrated a significant improvement in rectal sensation to distension. This suggests that sacral nerve stimulation has an effect on the afferent sensory fibres of the rectum. The contribution that this effect has to the clinical outcome is not known.

Improvement in urgency was a marked clinical finding in all patients. Urgency has previously been shown to relate to high-pressure bowel contractions[26], suggesting that this motor event may be altered by sacral nerve stimulation.

It is likely that sacral nerve stimulation involves an altered function ('neuromodulation') of all the nerve fibres within the sacral plexus, including somatic fibres to the external anal sphincter and pelvic floor, autonomic fibres to the internal anal sphincter and colon, and afferent sensory fibres from the anus and rectum. The relative contribution of each of these, and the central effect of stimulation on higher centres, remains to be elucidated.

In this series all patients had an intact, partially intact or surgically repaired external anal sphincter. This may be important in selecting future patients for sacral nerve stimulation, particularly as an increased squeeze pressure appears to be part of the underlying physiological mechanism. However, the results in patients with an anatomically disrupted external anal sphincter are unknown.

In all patients in this series it was possible to elicit the pudendal nerve latency on one or both sides. In previous studies patients with bilaterally absent pudendal nerves showed no benefit[13,24]; this raises the possibility that an impaired neural connection between the sacral plexus, anal sphincter and pelvic floor may lead to a poor clinical result. Patients with a complete spinal cord transection also appear not to benefit[27], while sacral nerve stimulation has been shown to be successful in patients with partial spinal lesions[17].

Sacral nerve stimulation provides an alternative approach to other therapies for faecal incontinence. However, its use should be restricted to patients who have failed maximum conservative therapies, and who experience severe incontinence. The authors have treated only a very small proportion of all incontinent patients referred to them with this technique.

This study has shown that patients resistant to maximal conventional treatment benefit from sacral nerve stimulation and that this benefit is maintained in the medium term. The morbidity is substantially less than with other surgical treatments and the percutaneous screening technique allows prediction of success, before surgical implantation. Studies are now required to determine the full range of indications and determine long-term follow-up.

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Original article

Consequences of axillary recurrence after conservative breast surgery

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Background: The aim was to study the incidence, time course and prognosis of patients who developed axillary recurrence after breast-conserving surgery, and to evaluate possible risk factors for axillary recurrence and prognostic factors after axillary recurrence.

Methods: In a population-based cohort of 6613 women with invasive breast cancer who had breast-conserving surgery between 1981 and 1990, 92 recurrences in the ipsilateral axilla were identified. Risk factors for axillary recurrence were studied in a case–control study nested in the cohort, and late survival was documented in the women with axillary recurrence.

Results: The overall risk of axillary recurrence was 1·0 per cent at 5 years and 1·7 per cent at 10 years. The risk of axillary recurrence increased with tumour size ($P = 0{\cdot}033$) and was highest in younger women (odds ratio (OR) 3·9 for women aged less than 40 years compared with those aged 50–59 years). Radiotherapy to the breast reduced the risk of axillary recurrence (OR 0·1 (95 per cent confidence interval 0·1 to 0·4)). The breast cancer-specific survival rate after axillary recurrence, as measured from primary treatment, was 78·0 per cent at 5 years and 52·3 per cent at 10 years. Tumour size and node status had a statistically significant effect on death from breast cancer.

Conclusion: Axillary recurrence is rare, although more common in younger women with large tumours. Radiotherapy to the breast was protective. Tumour size and node status were the most important prognostic factors in women with axillary recurrence.

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Introduction

Axillary recurrence is rare after breast-conserving surgery with a level I and II axillary dissection[1–8]. It can be followed by painful intractable growth into the brachial plexus; it is also associated with distant metastasis and death[7–9]. Some investigators claim that axillary recurrence is only an indicator of more aggressive disease[1,10], while others regard it as a possible source of distant dissemination[5,11].

A level I and II axillary dissection with removal of at least ten lymph nodes has been regarded as a reasonable compromise between the competing goals of accurate staging, regional control and postoperative complications[5]. Postoperative radiotherapy to the axilla reduces the risk of axillary recurrence[4,12]. However, in patients with between zero and three positive nodes after level I and II axillary dissection, axillary irradiation confers no or little benefit[2–6], and increases the risk of lymphoedema[3,5].

The number of retrieved and number of involved nodes are risk factors for axillary recurrence[1–4,6,10,13–16]. Patient age[1,8,17], histological grade[8], tumour size[1,9,18], presence of extracapsular extension of tumour[2,6] and location of the primary tumour[19] have also been implicated as possible risk factors. The prognosis in patients with axillary recurrence is poor, but survival estimates in most series have been difficult to interpret owing to the small number of patients.

In a cohort of 6613 women who had breast-conserving surgery for invasive breast cancer, 92 women with axillary recurrence were studied. The aim was to investigate the

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The Editors have satisfied themselves that all authors have contributed significantly to this publication

incidence and time course of axillary recurrence, and the prognosis in affected patients. A further aim was to evaluate possible risk factors for axillary recurrence and prognostic factors for breast cancer-specific survival.

Patients and methods

Incidence of axillary recurrence

A cohort of 6613 women with invasive breast cancer from five of Sweden's six healthcare regions, who had breast-conserving surgery between 1981 and 1990, was followed until 1997. Data were collected from regional breast cancer registers in three healthcare regions and from the medical records in the other two. The design of the cohort study has been described in detail[20], although with data on only 4694 women from four regions. The validity of the data from four regions was checked and found to be accurate, by detailed study of the records of 1332 controls, selected for a nested case–control study that aimed to analyse risk factors for local recurrence. In the fifth region the number of person-years was incorrect, and data from this region were excluded from the cohort analysis. The data on 4694 women from four regions with 107 axillary recurrences as a first event were therefore used in the analysis of the incidence of axillary recurrence.

Risk factors for axillary recurrence

Patients and three controls each were taken from the cohort of 6613 women, matched for year of primary operation and healthcare region. Ninety-two women with a local recurrence in the axilla before 1993 were included as index cases and 193 women who were free from a local or axillary recurrence up to the same interval at risk were included as controls. Three cases lacked eligible controls, so 88 of the 92 women were included in the analysis of risk factors for axillary recurrence.

Prognosis

The outcome for the 92 patients was analysed. The median follow-up for the women who had an axillary recurrence was 7·1 years. Twenty-three of these also had a simultaneous local recurrence in the breast.

Surgery

Routine axillary dissection included level I and II dissection[21] through a separate incision unless the primary breast tumour was situated high up in the axillary tail. During the 1980s, the regional treatment protocols recommended axillary dissection for all patients with invasive breast cancer. At a few of the participating centres patients had axillary sampling rather than axillary dissection. Some 8·1 per cent of the women had no axillary surgery. There was, however, a successively increasing compliance with treatment protocols over time[20].

Radiotherapy

Between 1981 and 1990 radiotherapy to the breast up to 50–58 Gy was standard treatment. Booster treatment was not used to any extent in Sweden. Regional radiotherapy of 46–50 Gy, generally including internal mammary, supraclavicular, infraclavicular and axillary nodes, was given to selected patients who did not have axillary surgery, those deemed to have undergone inadequate axillary surgery or those who were node positive.

Statistical analysis

The probability of axillary recurrence, and distant disease-free and breast cancer-specific survival was estimated with the life-table method[22]. The 5- and 10-year results are life-table estimates with standard errors. The differences between survival curves were tested with a log rank test[23].

To study the joint effect of different variables on prognosis, multivariate proportional hazards analyses[24] were computed. Since type of surgery, number of retrieved nodes and node status are closely related, in the multivariate model of prognostic factors, one at a time was used to determine which gave the best fit as judged by model χ^2 analysis. The estimates are given as relative hazard (RH) with 95 per cent confidence intervals.

Multivariate conditional logistic regression analyses were used to estimate the odds ratio and 95 per cent confidence intervals in the case–control study.

Results

Patient characteristics

Table 1 shows the characteristics of the 92 women with axillary recurrence compared with 193 matched controls from the nested case–control study. Women with axillary recurrence were younger, had larger tumours, and were more likely to have received axillary radiotherapy, but less likely to have had radiotherapy to the breast.

Incidence of axillary recurrence

Of 4694 patients, 107 developed an axillary recurrence as a first event. The overall risk of axillary recurrence was

1·0(0·1) per cent at 5 years and 1·7(0·2) per cent at 10 years. The probability of axillary recurrence was highest in women aged less than 40 years and tended to increase with increasing tumour size (*Table 2*). Women with an unknown axillary node status tended to have a higher probability of axillary recurrence than women with a known node status. The probability of axillary recurrence was higher in women who did not have radiotherapy to the breast.

Table 1 Characteristics of the 92 women with axillary recurrence who had breast-conserving surgery for invasive breast cancer between 1981 and 1990 compared with 193 controls from the source population

	Women with axillary recurrence	Controls
No. of patients	92	193
Age (years)*	58·2(14·6)	59·3(13·2)
Tumour size (mm)*	17·0(7·9)	14·1(6·9)
Axillary procedure‡		
Axillary dissection	55 (59·8)	146 (75·6)
Axillary sampling	26 (28·3)	34 (17·6)
No axillary surgery	11 (12·0)	13 (6·7)
Women with no retrieved nodes‡	6 (7·6)	7 (3·9)
No. of retrieved nodes*	6·0(4·6)	7·5(4·5)
Women with positive nodes‡	13 (16·0)	27 (14·9)
No. of positive nodes*	2·5(2·2)	2·3(1·9)
Other treatment‡		
Radiotherapy to the breast	39 (42·4)	130 (68·1)
Radiotherapy to the axilla	11 (12·0)	19 (9·8)
Adjuvant chemotherapy	2 (2·2)	4 (2·1)
Adjuvant hormonal therapy	15 (16·3)	34 (17·7)
Time to axillary recurrence (months)†	23·7 (1·5–103·5)	–
Time to death from breast cancer after axillary recurrence (months)†	29·8 (1·8–99·3)	–

*Values are mean(s.d.) or †median (range); ‡values in parentheses are percentages

Risk factors for axillary recurrence

The risk of axillary recurrence tended to diminish with age and to increase with tumour size (*Table 3*). An axillary dissection with retrieval of more than ten nodes tended to reduce the risk of recurrence in the axilla compared with axillary sampling or no axillary surgery. Radiotherapy to the breast was accompanied by a low risk of axillary recurrence. Age, tumour size and number of retrieved axillary nodes were analysed as continuous variables in a multivariate model. Trend tests gave a statistically significant result for tumour size ($P = 0.033$), but not for age ($P = 0.052$) or number of retrieved nodes ($P = 0.088$).

Prognosis in women with axillary recurrence

During follow-up, 43 of the 92 women with axillary recurrence died, 39 from breast cancer. Fifty women developed distant metastases. Seven of 92 were noted to have distant metastases within 1 month after the appear-

Table 2 Life-table estimates showing probability of remaining free from axillary recurrence in a cohort of 4694 patients who had breast-conserving surgery for invasive breast cancer between 1981and 1990

			Probability of no axillary recurrence (%)	
	No. of patients	No. with axillary recurrence	5 years	10 years
Age (years)				
< 40	314	14	97·1(1·0)	96·4(1·1)
40–49	1003	24	99·0(0·3)	98·5(0·4)
50–59	1117	18	99·2(0·3)	99·0(0·3)
60–69	1326	23	99·5(0·2)	98·5(0·4)
≥ 70	934	28	98·8(0·4)	97·5(0·5)
Tumour size (mm)*				
1–10	1641	20	99·6(0·2)	99·0(0·3)
11–20	2348	59	98·7(0·2)	98·3(0·3)
21–30	430	17	99·3(0·4)	96·7(0·9)
> 30	82	5	93·4(2·9)	–
Axillary node status				
Unknown	455	19	98·4(0·6)	97·0(0·9)
Negative	3510	74	99·0(0·2)	98·4(0·2)
Positive	729	14	99·1(0·4)	98·2(0·5)
Breast radiotherapy				
Yes	2730	48	99·3(0·2)	98·8(0·2)
No	1964	59	98·5(0·3)	97·6(0·4)

Values in parentheses are s.e. Analysis based on data from four healthcare regions. Owing to a longer follow-up in the study base, 107 patients with axillary recurrence were included in this analysis. *Data on tumour size were lacking for 193 patients

Table 3 Multivariate conditional logistic regression analysis of cases and controls from the source population who had breast-conserving surgery for invasive breast cancer between 1981 and 1990

	No. of cases*	No. of controls*	Odds ratio	*P* (trend test)
Age (years)				0·052
< 40	13	18	3·9 (1·1, 13·8)	
40–49	17	33	2·8 (0·9, 8·6)	
50–59	15	44	1·0	
60–69	21	62	1·3 (0·5, 3·5)	
≥ 70	22	34	0·6 (0·2, 1·7)	
Tumour size (mm)				0·033
1–10	15	64	0·5 (0·2, 1·1)	
11–20	49	105	1·0	
21–30	16	12	2·1 (0·8, 5·7)	
> 30	8	10	2·2 (0·7, 7·1)	
Surgery				
Axillary dissection	51	144	1·0	
Axillary sampling	26	34	2·2 (0·9, 5·1)	
No axillary surgery	11	13	3·0 (0·6, 16·0)	
Retrieved nodes				0·088
0–5	45	72	1·1 (0·5, 2·4)	
6–10	24	70	1·0	
> 10	6	35	0·4 (0·1, 1·4)	
Missing data	13	14		
Axillary node status				
Unknown	11	12	–	
Negative	66	152	1·0	
Positive	11	27	1·0 (0·2, 4·7)	
Breast radiotherapy				
Yes	35	130	0·1 (0·1, 0·4)	
No	53	61	1·0	
Axillary radiotherapy				
Yes	9	19	1·7 (0·2, 11·7)	
No	79	172	1·0	

Values in parentheses are 95 per cent confidence intervals. *Since four cases lacked invasive controls, and two controls had missing data about radiotherapy, 88 cases and 191 controls are included in the multivariate analysis

ance of the axillary recurrence, and 13 of 92 within 3 months.

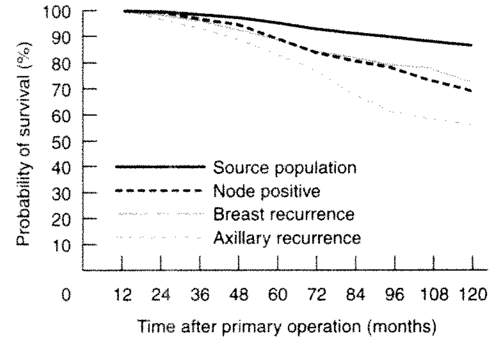
The life-table estimates for overall survival among women with axillary recurrence, as measured from primary treatment, were 77·0(4·4) per cent at 5 years and 48·4(6·0) at 10 years, while the breast cancer-specific survival estimates were 78·0(4·4) per cent at 5 years and 52·3(6·0) per cent at 10 years. The life-table curve for breast cancer-specific survival is shown in *Fig. 1*, together with the curves for the entire source population[20], node-positive women in the source population[20] and women with a local recurrence in the breast (unpublished data). Distant disease-free survival was 78·0(4·4) per cent at 5 years and 39·7(6·0) per cent at 10 years.

Life-table analysis of overall survival using follow-up after the appearance of recurrence instead of time from primary operation gave estimates of 59·2(5·3) per cent at 5 years and 43·5(6·8) per cent at 10 years, while the breast cancer-specific survival estimates were 62·0(5·3) per cent at 5 years and 48·2(6·8) per cent at 10 years. The distant disease-free survival after the appearance of axillary recurrence was 59·8(5·3) and 24·0(6·8) per cent at 5 and 10 years respectively.

Cox multivariate analysis of prognostic factors for breast cancer-specific survival showed that node status gave a better model fit as judged by model χ^2 analysis than the variables type of surgery and number of retrieved nodes (*Table 4*). Tumour size had a statistically significant effect on death in breast cancer (RH 2·0 (1·0 to 4·4)). The same was true for node status (RH 4·0 (1·1 to 14·4)).

Discussion

Axillary recurrence was reviewed in a large, unselected cohort of patients with breast cancer treated in many different centres. The case series is comparatively large,



No. at risk											
Source population	4694	4619	4503	4380	3830	3146	2412	1738	1247	927	587
Node positive	729	717	692	671	551	429	309	202	137	97	56
Breast recurrence	391	384	375	359	337	300	271	230	194	153	111
Axillary recurrence	92	89	86	81	76	67	55	46	36	27	18

Fig. 1 Life-table curve showing the estimated probability of breast cancer-specific survival, measured from the primary operation, in 92 women with axillary recurrence. Curves for the entire source population (*n* = 4694), node-positive women in the source population (*n* = 729) and women from the source population with local recurrence in the breast (*n* = 391) are shown for comparison

Table 4 Multivariate Cox proportional hazards analysis of prognostic factors for breast cancer-specific survival, as measured from primary operation, in 92 women with axillary recurrence who had breast-conserving surgery between 1981 and 1990

	No. of patients	Relative hazard for death from breast cancer
Age (years)		
< 50	31	0·5 (0·2, 1·1)
≥ 50	61	1·0
Tumour size (mm)		
≤ 20	66	1·0
> 20	26	2·0 (1·0, 4·4)
Node status*		
Unknown	11	–
Negative	68	1·0
Positive	13	4·0 (1·1, 14·4)
Breast radiotherapy		
Yes	39	1·3 (0·6, 2·6)
No	53	1·0
Axillary radiotherapy		
Yes	11	1·2 (0·3, 4·9)
No	81	1·0

Values in parentheses are 95 per cent confidence intervals. *Women who did not have axillary surgery were excluded, leaving only 81 patients for the full multivariate analysis

conferring sufficient statistical power for a study in this area. The validity of the background information was well controlled since large parts of the cohort information were collected through direct studies of original records; data from the randomly selected controls were thoroughly scrutinized.

All participating regions had treatment protocols for breast cancer and breast cancer collaborative groups. However, multidisciplinary breast cancer teams were established in only the larger hospitals, and the need for subspecialization in surgery and oncology was not recognized at the time. Thus, these results are applicable to situations of routine care, but may be better currently as a result of improved training of clinicians and more developed teamwork. Women with predominantly stage I or early stage II invasive cancer who had breast-conserving surgery were studied, and the results may not be generalized to women undergoing mastectomy or those with more advanced breast cancer.

The proportion of women with distant metastases shortly after the axillary recurrence was noteworthy. Possibly as a result of lengthy diagnostic assessment, these women may have been considered to have systemic disease at time of axillary recurrence.

These results can be discussed in at least two interesting clinical perspectives. First, the overall risk of axillary recurrence was low: less than 2 per cent overall and less than 4 per cent in any of the studied subgroups at 10 years. This implies that even a marked difference in odds ratio in the risk factor analysis represents low absolute risk differences, mostly around 2–3 per cent. Thus, more aggressive treatments specifically directed at reducing axillary recurrence would have to be weighed carefully in terms of benefits and risks, since the numbers needed to treat would be large.

Second, although the risk is low, an axillary recurrence causes significant suffering and presents a therapeutic dilemma. Since less extensive surgery, large tumour size and absence of radiotherapy are risk factors, the hypothesis emerges that most axillary recurrences in the present series were overlooked primary lymph node metastases. The finding that the traditional risk factors for death (node status, tumour size) also extend to patients with axillary recurrence is compatible with this theory. The sentinel node biopsy technique has brought new knowledge of relevance to this issue. The sentinel node, the first lymph node that drains the breast tumour, is usually situated in the lower part of the axilla, near or under the pectoral muscle[25,26]. It could be speculated that axillary dissection through a separate incision in the axilla in untrained hands might fail to explore the lowest part of the axilla. A positive sentinel node may therefore be left behind. In some women it may be sterilized by radiotherapy to the breast; in the multivariate analysis radiotherapy to the breast was clearly protective against axillary recurrence. Radiotherapy to the breast often includes the axillary tail and lower axilla[27]. In a study by Liljegren *et al.*[28] that compared the results of breast-conserving surgery in women who did or did not receive postoperative radiotherapy to the breast, regional and distant recurrence was more frequent in the group that did not receive radiotherapy. The difference between the groups was explained entirely by additional axillary recurrence in the non-irradiated group of women. If the sentinel node biopsy technique has the potential to reduce the frequency of overlooked primary lymph node metastases, this should reduce the incidence of axillary recurrence.

The finding concerning axillary radiotherapy in the present study should be interpreted cautiously. During this interval radiotherapy to the axilla was not standard treatment and was given only in special situations. Thus, the estimates may be influenced heavily by selection bias.

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Original article

Prognostic factors after oesophagectomy and extended lymphadenectomy for squamous oesophageal cancer

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Background: The prognosis of patients without nodal metastasis of oesophageal cancer is generally good, but recurrence develops in some cases.
Methods: Data on 88 consecutive patients with squamous oesophageal cancer who underwent three-field lymph node dissection from 1986 to September 1998 and who had no evidence of nodal disease were reviewed retrospectively. Disease status was based on histological examination of the section of each node with the largest surface area, stained with haematoxylin and eosin.
Results: The 3- and 5-year survival rates of patients without lymph node metastasis were 85 and 81 per cent respectively, better than in patients with metastasis. Twelve patients died from recurrence. Recurrence was haematogenous in nine patients and locoregional in three. Survival was worse in men, for patients with lesions located in the upper thoracic oesophagus, and in those with lymphatic or blood vessel invasion. Only the presence of lymphatic invasion correlated with survival on multivariate analysis ($P = 0.04$).
Conclusion: Although survival was generally good in patients without nodal metastasis from oesophageal cancer following three-field lymph node dissection, patients with lymphatic invasion remained at risk for haematogenous dissemination.

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Introduction

Lymph node dissection of the neck, mediastinum and abdomen has been practised routinely in Japan since the mid-1980s to reduce the high incidence of recurrence in cervical and superior mediastinal lymph nodes following oesophagectomy for oesophageal cancer[1,2]. The efficacy of this intervention remains controversial, but the extent of lymph node metastasis and its relationship to survival have been determined accurately. Patients without lymph node metastasis after extended lymphadenectomy achieve good survival, even when the oesophageal lesion has penetrated deeply. However, recurrence still occurs in some patients who have no evidence of disease in resected nodes. Therefore, it is essential to identify which patients remain at risk of recurrence after what was assumed to be a curative operation. This knowledge will improve the efficacy of follow-up and help to mould a strategy for prophylactic treatment. The determination of which clinicopathological features correlate with recurrence in patients without lymph node metastasis after three-field lymph node dissection was the subject of this investigation.

Patients and methods

The medical records of 247 consecutive patients with squamous cell carcinoma of the thoracic oesophagus, who had undergone oesophagectomy and lymphadenectomy of the neck, chest and abdomen in the authors' department from 1986 to September 1998, were reviewed. Patients with enlarged intra-abdominal para-aortic nodes on computed tomography, fixed cervical nodes or multiorgan involvement did not undergo three-field lymph node dissection, because these findings were considered to represent advanced disease that was not curable surgically. None of the patients had neoadjuvant treatment. All patients had a standard three-stage oesophagectomy and three-field lymph node dissection[3]. The diagnosis of metastasis was based on histological examination of the section of lymph node with the maximum surface area, stained with haematoxylin and eosin.

A total of 159 patients was proven to have metastasis in resected nodes, and the other 88 patients (74 men and 14 women; mean age 63 (range 43–83) years) did not. None of the patients with three or fewer metastasized nodes had adjuvant treatment. The patients who had four or more metastasized nodes had adjuvant chemotherapy with 5-fluorouracil and cisplatin after recovery from the operation. Therefore, surgery was the sole treatment received by patients without nodal involvement. The clinicopathological features, including location, size, histological classification, depth of invasion, growth pattern of the oesophageal lesion and presence of vascular invasion, were classified according to guidelines from the Japanese Society for Oesophageal Disease[4].

The median length of follow-up for the 88 patients with no lymph node metastasis was 74 months, excluding 12 patients who died from causes unrelated to recurrence and two in-hospital deaths (2 per cent).

Table 1 Clinicopathological characteristics and prognosis of 247 patients who had curative resection for oesophageal cancer

	No. of patients	Survival rate (%)		
		3 years	5 years	*P**
Overall		55·3	50·6	
Sex				0·34
Male	195	52·1	47·7	
Female	52	66·6	61·0	
Age (years)				0·83
< 65	137	54·2	44·6	
≥ 65	110	57·0	57·0	
Location of tumour				0·02
Upper thoracic oesophagus	28	44·7	44·7	
Middle thoracic oesophagus	149	65·2	55·7	
Lower thoracic oesophagus	70	39·3	39·3	
Depth of tumour invasion				< 0·001
pTis, pT1	65	88·6	83·9	
pT2	45	66·8	61·3	
pT3, pT4	137	37·7	33·0	
Histological classification				0·91
Well differentiated	40	64·5	52·1	
Moderately differentiated	125	52·1	48·4	
Poorly differentiated	82	59·9	55·9	
Infiltrative growth pattern				0·02
Expansive	48	63·1	56·1	
Intermittent	169	56·1	56·1	
Invasive	30	34·4	17·2	
No. of positive nodes				< 0·001
0	88	85·0	81·0	
1–3	79	48·8	47·5	
≥ 4	80	24·4	15·1	
Lymphatic invasion				< 0·001
Positive	161	41·8	36·7	
Negative	86	79·0	75·9	
Vascular invasion				0·03
Positive	76	38·9	38·9	
Negative	171	62·3	56·4	

pTis, carcinoma *in situ*; pT1, tumour invading mucosal or submucosal layer only; pT2, tumour invasion into muscularis propria; pT3, tumour invasion to adventitia; pT4, contiguous spread into surrounding structures. *Wilcoxon test

The relationships between survival and clinicopathological features were analysed with the StatView® 4.51 J program (ABACUS Concept, Berkeley, California, USA). Survival was calculated by the Kaplan–Meier method, and the significance of differences in survival was evaluated by the generalized Wilcoxon test. The Cox proportional hazard model was used to determine the clinicopathological factors affecting survival, and the χ^2 test to analyse relationships between the presence of lymph node metastasis and lymphatic and blood vessel invasion. $P < 0·05$ was considered significant.

Results

The overall 3- and 5-year survival rates for the 247 patients who underwent oesophagectomy were 55 and 51 per cent respectively. The mean(s.d.) number of nodes dissected in each patient was 61(25). The survival rate of patients without nodal metastasis was better at 3 and 5 years (85 and 81 per cent respectively) than in patients with nodal metastasis (40 and 32 per cent respectively) ($P < 0·001$). The 5-year survival rate was inversely related to the number of positive nodes: 48 per cent in patients with one to three positive nodes and 15 per cent in those with four or more positive nodes. Tumour location, depth of tumour invasion, infiltrative growth pattern, lymphatic invasion and blood vessel invasion also correlated with survival (*Table 1*).

Table 2 Cox regression analysis of 247 patients who had curative resection for oesophageal cancer

Prognostic factor	Relative risk	*P*
Location of tumour in thoracic oesophagus		0·42
Upper third	1	
Middle third	0·67 (0·29, 1·55)	
Lower third	0·91 (0·38, 2·18)	
Depth of tumour invasion		0·02
pTis, pT1	1	
pT2	2·87 (0·99, 8·32)	
pT3, pT4	3·47 (1·42, 8·46)	
No. of positive nodes		< 0·001
≥ 4	1	
1–3	0·18 (0·09, 0·38)	
0	0·58 (0·34, 0·99)	
Lymphatic invasion		0·22
Positive	1	
Negative	0·67 (0·36, 1·26)	
Vascular invasion		0·84
Positive	1	
Negative	0·95 (0·56, 1·62)	

Values in parentheses are 95 per cent confidence intervals. pTis, carcinoma *in situ*; pT1, tumour invading mucosal or submucosal layer only; pT2, tumour invasion into muscularis propria; pT3, tumour invasion to adventitia; pT4, contiguous spread into surrounding structures

Simultaneous multivariate analysis by the Cox model, using location of tumour, depth of invasion, lymphatic and blood vessel invasion, and number of positive nodes, showed that the number of positive nodes was the factor that correlated most closely with survival (*Table 2*).

The clinicopathological features of patients without nodal involvement are shown in *Table 3*. The male : female ratio was 5·3, and survival was worse in men than in women. Survival in patients with a lesion in the upper third of the thoracic oesophagus was worse than that in other patients. Lymphatic invasion was identified in 36 patients (41 per cent) and blood vessel invasion in 16 (18 per cent). The incidence of lymphatic and blood vessel invasion in patients with lymph node metastasis was 79 and 38 per cent respectively (*Table 4*). Invasion to the level of the muscularis propria, lymphatic invasion and blood vessel invasion all had a negative effect on survival, but the size of the primary lesion did not (*Table 3*).

Twelve patients died from recurrence. The depth of invasion was to the submucosal layer in three patients, to the muscularis propria in one patient, and reached the adventitia in eight patients. Lymphatic invasion was identified in nine patients. The pattern of recurrence was haematogenous in nine patients (liver, four; lung, three; bone, two) and locoregional in three patients. Lymphatic recurrence did not occur.

Table 3 Clinicopathological characteristics and prognosis of patients with no evidence of nodal metastasis of oesophageal cancer

	No. of patients	Survival rate (%)		P*
		3 years	5 years	
Sex				< 0·001
Male	74	81·9	74·4	
Female	14	100	100	
Age (years)				0·22
< 65	47	89·9	84·9	
≥ 65	41	79·5	73·8	
Location of tumour				0·02
Upper thoracic oesophagus	13	58·2	58·2	
Middle thoracic oesophagus	54	89·9	81·3	
Lower thoracic oesophagus	21	89·2	89·2	
Depth of tumour invasion				0·05
pTis, pT1	43	92·4	85·3	
pT2	12	88·9	88·9	
pT3, pT4	33	75·1	69·7	
Maximum tumour diameter (cm)				0·68
< 4·9	56	83·9	79·7	
≥ 5	26	90·5	81·4	
Unknown	6			
Histological classification				0·72
Well differentiated	18	88·9	74·1	
Moderately differentiated	44	83·6	83·6	
Poorly differentiated	26	84·9	76·4	
Infiltrative growth pattern				0·29
Expansive	30	86·6	75·8	
Intermittent	53	82·4	77·3	
Invasive	5	66·7	66·7	
Lymphatic invasion				0·004
Positive	36	72·1	66·9	
Negative	52	94·9	89·6	
Vascular invasion				0·027
Positive	16	66·6	66·6	
Negative	72	89·4	83·0	

pTis, carcinoma *in situ*; pT1, tumour invading mucosal or submucosal layer only; pT2, tumour invasion into muscularis propria; pT3, tumour invasion to adventitia; pT4, contiguous spread into surrounding structures. *Wilcoxon test

Table 4 Relationship between the incidence of lymphatic and vascular invasion and the incidence of lymph node metastasis

	Lymph node metastasis		P*
	Present	Absent	
Lymphatic invasion			< 0·001
Positive	125 (79)	36 (41)	
Negative	34 (21)	52 (59)	
Vascular invasion			< 0·002
Positive	60 (38)	16 (18)	
Negative	99 (62)	72 (82)	

Values in parentheses are percentages. *χ^2 test

Table 5 Cox regression analysis of data from patients with no evidence of nodal metastasis of oesophageal cancer on routine histological examination

	Univariant		Multivariant	
	Relative risk	P	Relative risk	P
Location of tumour	—	0·04	—	0·17
Lower	0·15 (0·03, 0·86)	0·03	0·18 (0·02, 1·25)	0·82
Middle	0·24 (0·07, 0·86)	0·03	0·32 (0·08, 1·28)	0·11
Lymphatic invasion (positive, negative)	4·92 (1·34, 18·08)	0·02	4·42 (1·11, 17·60)	0·04
Vascular invasion (positive, negative)	3·52 (1·14, 10·87)	0·03	1·36 (0·36, 5·12)	0·65

Values in parentheses are 95 per cent confidence intervals

Univariate analysis of these clinicopathological factors showed that tumour location, lymphatic invasion and blood vessel invasion affected the survival of patients without nodal metastasis who underwent oesophagectomy. Multivariate analysis showed that lymphatic invasion was the only factor that correlated with survival (*Table 5*).

Discussion

In Japan, oesophagectomy with three-field (neck, mediastinum and abdomen) lymph node dissection is performed routinely as a curative procedure in patients with oesophageal cancer[5,6]. Since 1986, the authors' department has routinely combined three-field lymph node dissection with oesophagectomy to treat patients with potentially curative oesophageal cancer (no multiorgan involvement, fixed cervical lymph nodes or enlargement of the intra-abdominal para-aortic nodes on computed tomography[7]). Hospital mortality and pulmonary morbidity rates were 5 and 16 per cent respectively[7,8]. Fujita *et al.*[5] reported that patients with upper or middle thoracic oesophageal carcinoma with lymph node metastasis had a better survival rate after three-field than following two-field lymph node dissection. The effect on survival of prophylactic systemic lymph node dissection remains controversial, because most studies of three-field lymph node dissection are retrospective and not randomized. What is clear, however, is that the more extensively the nodes are dissected, the more precisely the disease can be staged. It has been reported that the 5-year survival rate of patients without nodal disease is 50 per cent or less when particular attention is not paid to lymph node dissection, whereas the survival rate was 82 per cent after three-field lymph node dissection[7–9]. This difference was not only the result of the curative effect of lymph node dissection, but also of reducing stage migration. As the presence of metastasis cannot be diagnosed based on size or shape[3], thorough three-field lymph node dissection is the only reliable way to stage oesophageal cancer accurately.

Previous investigators have identified a variety of factors that affect survival after extended lymph node dissection[5,10]. Depth of tumour invasion, location of lymph node metastases and degree of tumour differentiation are well established factors that influence survival. Among these, the number of positive nodes has the highest correlation with recurrence[7,11].

Hayashi *et al.*[12] demonstrated that genetic examination of *p53* and K-*ras* mutations was more sensitive than conventional histological examination in detecting occult lymph node metastasis in colorectal cancer. However, the incidence of the *p53* mutation in oesophageal cancer is much lower than in colorectal cancer, decreasing its value as a tumour marker[13,14]. Immunohistochemical staining with anticytokeratin antibodies has also been reported to be more sensitive than conventional histological examination in identifying tumour. As conventional histological examination was used in the present study, it is possible that some occult disease was missed. However, the diagnoses were remarkably accurate, because the 5-year survival rate was very different between patients diagnosed as having and not having nodal metastasis. This is of practical importance because it is impossible routinely to perform immunohistochemical staining of all the serial sections of resected nodes in all patients to avoid missing micrometastases. It is of further reassurance to note that Glickman *et al.*[15] found no correlation between the presence of micrometastasis and a poor outcome. However, both Komukai *et al.*[16] and Natsugoe *et al.*[17] reported the contrary. The clinical significance of micrometastasis remains to be determined. The purpose of the present study was not to detect micrometastasis but to define the relationship between pathological factors that relate to survival in patients who have no evidence of nodal involvement. The data unequivocally demonstrate that poor outcome is closely correlated with lymphatic invasion.

Lymphatic and blood vessel invasion both correlated with lymph node metastasis. The significance was greater for lymphatic invasion than for blood vessel invasion. Both variables showed high specificity for lymph node metastasis, although the sensitivity of lymphatic invasion was higher. Lymphatic invasion also related closely with systemic spread, even in patients without lymph node metastasis. Here again, it might be hypothesized that micrometastasis was likely to be present in patients with lymphatic invasion. Luketich *et al.*[18] detected micrometastasis in histologically negative lymph node specimens of oesophageal cancer using sophisticated methods, such as a reverse transcriptase–polymerase chain reaction assay to detect carcinoembryonic antigen messenger RNA. Five of ten patients had micrometastasis even though the pathological stage was N0. Two patients with and one patient without micrometastasis died from recurrence. In the present study, 12 patients with no histological evidence of nodal disease died from recurrence. Nine had lymphatic invasion, representing 25 per cent of the 36 similar patients, whereas only three patients without lymphatic invasion died. This represented only 6 per cent of 52 patients who did not have lymphatic invasion. The predictive value of lymphatic invasion for systemic dissemination in patients with histologically negative lymph nodes is similar to that predicted by Luketich *et al.*[18].

In conclusion, although survival was generally good in patients with node-negative oesophageal cancer after three-field lymph node dissection, patients who have lymphatic invasion are at high risk of haematogenous spread and require close follow-up.

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Original article

Cohort study in South and West England of the influence of specialization on the management and outcome of patients with oesophageal and gastric cancers

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Background: To evaluate specialization in National Health Service (NHS) cancer care, volume–outcome relationships were examined.
Methods: This was a cohort study of 1512 patients with oesophageal or gastric cancer in 23 acute NHS hospitals. Outcomes were survival time and operative (30 day) mortality. Multiple regression analysis was performed, adjusted for diagnoses, prognoses and treatments.
Results: For oesophageal cancer, the operative mortality rate decreased by 40 per cent (odds ratio 0·60 (95 per cent confidence interval (c.i.) 0·36 to 0·99 per cent); $P = 0{\cdot}047$) for each increase of ten patients in doctors' annual surgical caseloads, and the risk of death decreased by 8 per cent (hazard ratio 0·92 (95 per cent c.i. 0·85 to 0·99); $P = 0{\cdot}021$) for each increase of ten patients in doctors' annual caseloads. For gastric cancer, the operative mortality rate decreased by 41 per cent (odds ratio 0·59 (95 per cent c.i. 0·32 to 1·07)) for each increase of ten patients in doctors' annual surgical caseloads, and the risk of death decreased by 7 per cent (hazard ratio 0·93 (95 per cent c.i. 0·89 to 0·98); $P = 0{\cdot}009$) for each increase of ten patients in hospitals' annual caseloads. Patients of higher-volume doctors were more likely to receive most investigations and treatments, independently of presenting features.
Conclusion: The study supports concentration of services for oesophageal and gastric cancers. Specialization of doctors and their teams is at least as important as specialization of hospitals.

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Introduction

The mortality rate for oesophageal and gastric cancer is higher in the UK than in most other European countries[1]. It has been assumed that this largely reflects non-specialist care and has led to plans for the reorganization of cancer services in the UK[2]. Evidence of improved outcome linked to specialist care for specific types of cancer is, however, generally absent or inconsistent[3–5]. Evidence of advantages related to specialist care exists for breast cancer[6,7], ovarian cancer[8–10] and malignant teratoma[11] in the UK, while evidence for colorectal cancer is inconsistent[12,13]. There is no evidence of comparable quality for oesophageal and gastric cancers, which comprise over 9 per cent of all cancers in England and Wales[14].

Several studies into oesophageal and gastric cancer have focused on surgical (operated) patients in an effort to link outcome to experience, either on the part of the institution or the surgeon. An English cancer registry study of oesophagectomies in the late 1980s found no independent association between operative mortality rate and hospital surgical volume[15], although two recent North American studies have demonstrated a lower mortality rate for oesophagectomy at 'high-volume' compared with 'low-volume' hospitals[16,17].

Small UK and North American studies have also revealed that the operative mortality rate is lower among high-

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The Editors have satisfied themselves that all authors have contributed significantly to this publication

volume surgeons[18,19]. A large audit of oesophageal and gastric cancer in Wales also demonstrated a lower mortality rate (12 per cent) among surgeons who carried out six or more operations per year, compared with those doing fewer than this (17 per cent)[20]. For gastric cancer, clear evidence of benefit from specialization is lacking[3,4]. A study of 69 hospitals in the USA in 1977 found no independent association between in-hospital mortality rate and doctors' or hospitals' patient volumes[21], although in the Welsh audit higher surgical volumes were associated with a lower mortality rate after abdominal operations[20]. All of these studies are, however, open to the major criticism that confounding factors (e.g. case-mix) have not been taken into consideration.

There have been no cohort studies of volume–outcome relationships in oesophageal or gastric cancer care that include the full clinical spectrum, account for prognostic factors (such as stage of disease or co-morbidity), consider the impact of various investigative modalities and treatments, follow survival beyond hospital and use robust indicators of specialization. The aims of this study were to investigate relationships between specialization and patterns of clinical practice (use of tests, choice of treatment) and to estimate the independent effects of specialization on overall survival and operative mortality after controlling for confounding variables.

Patients and methods

The study population comprised all patients diagnosed for the first time as having oesophageal or gastric cancer in all 23 acute National Health Service (NHS) hospital trusts in the former South and West region of England during 1 year, from July 1996 to June 1997. Patients were identified prospectively in each hospital as the study progressed, primarily by regularly examining discharge and pathology diagnostic codes. Additional sources, such as departmental clinical information systems, were used as appropriate for each hospital.

Patient data were extracted from hospital records by three trained researchers. Unfamiliar terms were catalogued and discussed with the clinician responsible for this training, then coded appropriately to minimize error. Records were examined as immediately as possible and a year later. Any key prognostic variables that were missing were coded as unknown. If specific symptoms, co-morbid conditions, tests or treatments were not recorded, they were coded as absent, as these variables were generally recorded only if present. Histological evidence, when available, from resected specimens was used for staging; if not, other test results were used.

Specialization was indicated by doctors' and hospitals' patient volumes. Each patient's main doctor and main hospital trust were defined according to where the patient received their main treatment, in the following order of precedence: surgery, stent, radiotherapy and chemotherapy. For patients who received none of these treatments, their first hospital and consultant were their main hospital and doctor. 'Hospital' and 'doctor' volumes represented the numbers of patients with the respective cancer managed by their main hospital or main doctor during the year, using the system above. For analysis of operative mortality, doctors' and hospitals' surgical (operative) volumes were used. The surgeon was defined as the individual who carried out the patient's main operation in the order of precedence: resection, bypass, and 'open and close'.

For each cancer site a subgroup of junctional tumours was identified[22,23]. Among oesophageal cancers, junctional tumours were defined as any adenocarcinomas with an upper margin at least 35 cm from the incisors, or any tumour recorded as being a junctional adenocarcinoma at postoperative pathological examination. Among gastric cancers, junctional tumours were defined as any adenocarcinoma designated as 'cardia' by either the surgeon or pathologist[23].

Operative mortality was defined as death within 30 days of an operation. Each patient's survival was tracked with the NHS Central Register, for between 25 and 37 months or until death. The Register records all deaths in England and Wales daily. Townsend and Carstairs area deprivation scores were derived from each patient's postal code, linked to enumeration district data from the 1991 census[24].

Statistical analysis aimed to estimate the effect of doctor and hospital volumes on the use of investigations, choice of treatment, overall survival and operative mortality rate. Potential confounding was investigated first by examining relationships between prognostic variables, tests, treatments, doctor and hospital volumes, and outcomes. Doctor and hospital volumes had skewed distributions. Influences on operative mortality rate were examined using multiple logistic regression, and influences on survival time were examined with Cox's proportional hazards model. Using Stata statistical software[25], all potentially influential prognostic variables, doctor and hospital volumes, tests and treatments were entered into the initial models. Prognostic variables included age, sex, symptoms (weight loss, dysphagia and intestinal bleeding), co-morbidities (ischaemic heart disease, chronic obstructive airway disease, diabetes and hypertension), initial haemoglobin and albumin levels, use of diagnostic and staging investigations, tumour histology, node involvement, distant metastases, cancer stage, junctional tumours, emergency admission, treatments provided, deprivation scores and, for operative mortality, the American Society of Anesthesiologists (ASA) grade. Stratified analysis was used to control for confounding by variables with non-proportional hazards[26].

Doctor and hospital volumes were examined primarily as continuous variables. Potential interactions between types of treatment and hospital and doctor volumes were examined, as planned before. To convert the adjusted odds ratios or hazard ratios associated with a volume difference of one to ratios for larger volume differences, they were exponentiated to the power of the volume differences. Sensitivity analyses were conducted to assess the sensitivity of odds ratios and hazard ratios for doctor and hospital volumes to misclassification of the stage of unstaged patients. Patients with unknown stage were reclassified, first as having potentially curable disease (stages I–III), and then as having incurable disease (stage IV). To assess the influence on the regression results of potential clustering of outcomes among patients of the same doctor or hospital, the analyses were repeated using the Huber–White robust estimation 'cluster' procedure available in Stata[25].

The study was approved by the relevant multicentre and local research ethics committees. Further details of methods and results are available from the authors.

Results

Doctor and hospital volumes

Some 781 patients with oesophageal cancer and 731 patients with gastric cancer were identified in 23 hospital trusts. The distribution of patients by doctor and hospital volumes is shown in *Table 1*. Half of oesophageal patients and three-quarters of gastric patients were managed by doctors who dealt with less than one new patient with the respective cancer per month. Of patients who had surgery, 172 (53 per cent) of 322 patients with oesophageal cancer and 283 (70 per cent) of 405 with gastric cancer were operated on by surgeons who operated on less than one new patient per month with the respective cancer. A third (235 of 727) of all surgical patients were operated on by surgeons who performed less than one oesophageal or gastric cancer operation per month.

Table 1 Distribution of patients by doctor and hospital volumes

	Doctor volume	Hospital volume
Oesophageal cancer		
Total volume	12 (1–48; 6–25)	42 (13–83; 24–66)
Surgical volume*	10 (1–40; 6–19)	21 (1–56; 10–40)
Gastric cancer		
Total volume	7 (1–22; 3–12)	38 (9–67; 25–60)
Surgical volume*	8 (1–20; 4–13)	19 (1–39; 15–31)
Oesophageal or gastric cancer		
Total volume	18 (1–50; 6–29)	79 (4–145; 49–128)
Surgical volume*	18 (1–42; 9–23)	35 (10–87; 22–49)

Values are median (range; interquartile range). *Patients who underwent an operation

Case-mix

For oesophageal cancer, no symptoms or co-morbid conditions were associated with doctor or hospital volume. Patients of higher-volume hospitals and doctors were significantly less likely to be admitted as an emergency ($P = 0.04$ and $P < 0.001$ respectively). Patients of higher-volume doctors were significantly more likely to have junctional tumours ($P = 0.01$) but there was no such association with hospital volume ($P = 0.33$). Cancer stage was associated with doctor ($P < 0.001$) but not hospital ($P = 0.11$) volume. Patients of higher-volume doctors were more likely to have stage I–III disease.

For gastric cancer, patients of higher-volume doctors tended to be younger ($P < 0.001$) and were less likely to be admitted as an emergency ($P < 0.001$). Patients of higher-volume doctors were more likely to have stage I–III disease and less likely to have metastases or completely unstaged disease ($P < 0.001$). Patients of higher-volume hospitals were more likely to have metastases ($P = 0.03$).

Investigations and choice of treatment

Most patients had endoscopy and biopsy, but the provision of other investigations varied widely (*Table 2*). Patients of higher-volume doctors were significantly more likely to undergo various investigations, even after adjusting for prognostic factors known at the time of presentation.

For both cancers, about a third of patients had a resection, which was more likely with earlier stages of disease (*Tables 3* and *4*). After controlling for prognostic and clinical features, with both types of cancer patients of higher-volume doctors were more likely to undergo resection and less likely to receive none of the treatments considered (*Table 5*). Among patients with gastric cancer who underwent surgery, 'open and close' laparotomies were less likely ($P = 0.05$) with increasing doctors volume. Patients with oesophageal cancer of higher-volume doctors were more likely to have chemotherapy and were less likely to have radiotherapy, and patients with gastric cancer were more likely to have bypass surgery (*Table 5*). Patients with oesophageal cancer in higher-volume hospitals were more likely to have radiotherapy or dilatation; hospital volumes were not independently associated with any other type of treatment.

Table 2 Influence of doctor and hospital volumes on provision of investigations: logistic regression models

Investigation	Prevalence (%)	Odds ratio* Doctor volume	 Hospital volume
Oesophageal cancer			
Endoscopy	96	1·29 (0·91, 1·81)	0·92 (0·76, 1·14)
Biopsy	89	1·08 (0·88, 1·33)	0·99 (0·88, 1·13)
Computed tomography	53	1·38 (1·21, 1·58)	0·90 (0·83, 0·98)
Contrast swallow	51	0·91 (0·82, 1·03)	0·98 (0·91, 1·05)
Abdominal ultrasonography	21	0·97 (0·85, 1·10)	1·06 (0·98, 1·15)
Laparoscopy	6	0·78 (0·60, 1·04)	1·04 (0·89, 1·21)
Endoscopic ultrasonography	5	1·68 (1·21, 2·32)	0·69 (0·56, 0·84)
Gastric cancer			
Endoscopy	90	1·61 (1·03, 2·52)	1·01 (0·87, 1·17)
Biopsy	87	1·60 (1·08, 2·37)	1·03 (0·90, 1·17)
Abdominal ultrasonography	42	1·28 (0·99, 1·66)	1·08 (0·98, 1·20)
Computed tomography	40	0·93 (0·71, 1·21)	1·05 (0·95, 1·16)
Contrast swallow	25	0·90 (0·64, 1·17)	1·07 (0·95, 2·81)
Laparoscopy	12	3·13 (2·06, 4·77)	1·22 (1·02, 1·45)
Endoscopic ultrasonography	3	8·32 (2·35, 19·13)	0·93 (0·52, 1·68)

Values in parentheses are 95 per cent confidence intervals. *Odds ratio for a volume difference of ten patients. Each model contains the respective test as outcome, and has doctor volume, hospital volume and influential clinical features as explanatory variables

Table 3 Percentage of patients with oesophageal cancer who received specific treatments, by doctor and hospital volume tertiles

		Hospital volume tertile				Doctor volume tertile			
Treatment	Total (*n* = 781)	Low (7–32)* (*n* = 269)	Medium (35–53)* (*n* = 218)	High (60–83)* (*n* = 279)	*P*†	Low (1–6)* (*n* = 240)	Medium (7–14)* (*n* = 207)	High (17–48)* (*n* = 255)	*P*†
Resection	31	26	30	38	< 0·001	11	29	56	< 0·001
Stent	38	44	36	35	0·03	42	41	32	0·04
Dilatation	37	29	37	46	< 0·001	30	37	41	0·06
Chemotherapy	11	5	15	13	< 0·17	5	15	13	0·007
Radiotherapy	24	14	32	36	< 0·001	25	32	9	< 0·001
None of the above treatments	10	15	10	6	< 0·001	22	6	4	< 0·001

*Numbers per year are shown in parentheses. †*P* value from logistic regression using continuous volume variable, without adjusting for co-variates

Table 4 Percentage of patients with gastric cancer who received specific treatments, by doctor and hospital volume tertiles

		Hospital volume tertile				Doctor volume tertile			
Treatment	Total (*n* = 731)	Low (9–26)* (*n* = 233)	Medium (31–40)* (*n* = 256)	High (44–67)* (*n* = 233)	*P*†	Low (1–4)* (*n* = 251)	Medium (5–9)* (*n* = 207)	High (10–12)* (*n* = 255)	*P*†
Resection	37	42	36	31	0·01	18	34	57	< 0·001
Bypass	5	6	5	5	0·83	3	5	7	0·01
Stent	3	5	0	5	0·80	1	6	3	0·87
Chemotherapy	11	8	17	9	0·91	2	4	2	0·85
Radiotherapy	2	2	4	2	0·94	2	4	2	0·57
None of the above treatments	48	44	46	53	0·09	69	42	31	< 0·001

*Numbers per year are shown in parentheses. †*P* value from logistic regression using continuous volume variable, without adjusting for co-variates

Table 5 Influences of doctor and hospital volumes on treatment provision: logistic regression models

Treatment	Prevalence (%)	Odds ratio*	
		Doctor volume	Hospital volume
Oesophageal cancer			
Resection	31	1·71 (1·45, 2·00)	0·98 (0·89, 1·08)
Stent	38	1·03 (0·90, 1·18)	0·94 (0·87, 1·02)
Dilatation	37	1·03 (0·90, 1·18)	1·14 (1·04, 1·23)
Radiotherapy	24	0·71 (0·61, 0·84)	1·33 (1·21, 1·48)
Chemotherapy	11	1·29 (1·03, 1·60)	1·01 (0·88, 1·16)
None of the above treatments	10	0·46 (0·32, 0·69)	0·87 (0·75, 1·01)
Gastric cancer			
Resection	37	2·59 (1·82, 3·67)	0·98 (0·86, 1·12)
Bypass procedure	5	2·12 (1·23, 3·64)	1·05 (0·83, 1·33)
Stent	3	0·85 (0·39, 1·84)	1·05 (0·80, 1·37)
Chemotherapy	11	0·69 (0·44, 1·08)	1·04 (0·89, 1·22)
Radiotherapy	2	0·62 (0·26, 1·48)	1·05 (0·78, 1·40)
None of the above treatments	48	0·52 (0·37, 0·72)	1·02 (0·90, 1·15)

Values in parentheses are 95 per cent confidence intervals. *Odds ratio for a volume difference of ten patients. Each model contains the respective test as outcome. and has doctor volume, hospital volume and influential clinical features as explanatory variables

Operative mortality

Of the 322 patients with oesophageal cancer who had surgery, 36 (11 per cent) died within 30 days of operation. The operative mortality rate was lower with increasing surgeons' volume ($P = 0{\cdot}01$), and was lower but not significantly associated with increasing hospitals' surgical volume ($P = 0{\cdot}057$). In the multiple logistic regression model, operative death was about 5 per cent less likely for each extra patient treated by a surgeon per year (odds ratio 0·95 (95 per cent confidence interval (c.i.) 0·90 to 1·00); $P = 0{\cdot}047$), after adjusting for hospitals' surgical volume, age, stage, ASA score, ischaemic heart disease and resection *versus* other operation (*Table 6*).

Of the 405 patients with gastric cancer who had surgery, 55 (14 per cent) died within 30 days. In the multiple logistic regression model, the operative mortality rate was lower with increasing doctor volume (*Table 6*).

Since many surgeons perform both oesophageal and gastric cancer operations, oesophageal and gastric surgical patients were examined together. The operative mortality rate was significantly lower ($P = 0{\cdot}002$) with increases in doctors' pooled oesophagogastric surgical volumes (*Table 6*).

Sensitivity analyses showed the primary analyses to be robust.

Survival time

All but two patients were matched with the NHS Central Register. All survivors were followed for at least 2 years (range 25–41, median 31 months).

Table 6 Influences of doctors' surgical volumes on operative mortality: logistic regression models

Difference in doctors' surgical volumes	Odds ratio*	
	Crude	Adjusted†
Oesophageal cancer		
1	0·95 (0·92, 0·99)	0·95 (0·90, 1·00)
10	0·61 (0·42, 0·89)	0·60 (0·36, 0·99)
20	0·38 (0·18, 0·79)	0·35 (0·13, 0·99)
40	0·14 (0·03, 0·63)	0·13 (0·02, 0·97)
Gastric cancer		
1	0·95 (0·90, 1·00)	0·95 (0·89, 1·01)
10	0·60 (0·35, 1·04)	0·59 (0·32, 1·07)
20	0·36 (0·12, 1·09)	0·34 (0·10, 1·14)
Oesophageal or gastric cancer		
1	0·97 (0·95, 0·99)	0·97 (0·94, 0·99)
10	0·73 (0·59, 0·89)	0·70 (0·56, 0·88)
20	0·53 (0·35, 0·80)	0·49 (0·31, 0·78)
40	0·28 (0·12, 0·63)	0·24 (0·10, 0·60)

Values in parentheses are 95 per cent confidence intervals. *Odds ratio for volume difference of one exponentiated to power of the respective volume difference. †For oesophageal cancers, adjusted for hospital surgical volume, age, ischaemic heart disease, cancer stage, American Society of Anesthesiologists (ASA) grade, and resection *versus* other operation. For gastric cancers, adjusted for hospital surgical volume, age, albumin level and ASA grade. For oesophageal or gastric cancers combined, adjusted for hospital surgical volume, age, albumin level, ASA grade, resection *versus* other operation, tumour site (oesophageal, oesophageal junctional, gastric, gastric junctional). Other variables examined for possible inclusion in the model: emergency admission, weight loss, dysphagia, vomiting, co-morbidity, chemotherapy, radiotherapy

For oesophageal cancer, before adjustment for confounding, survival time was longer with higher doctor volume, higher hospital volume, younger age, no reported

Table 7 Predictors of survival time for all patients with oesophageal cancer: Cox's proportional hazards models

		Hazard ratio†			
Explanatory variable	*n**	Crude	Adjusted for case-mix, and hospital and doctor volumes	Adjusted for case-mix, hospital and doctor volumes, and treatment§	*P*§
No. of patients	750 (100)				
Doctor volume‡		0·82 (0·76, 0·87)	0·89 (0·83, 0·95)	0·92 (0·85, 0·99)	0·021
Hospital volume‡		0·95 (0·91, 0·99)	0·99 (0·95, 1·03)	1·01 (0·96, 1·05)	0·74
Age (years)‡		1·29 (1·21, 1·38)	1·14 (1·05, 1·23)	1·07 (0·98, 1·16)	0·14
Emergency admission	170 (23)	1·74 (1·46, 2·09)	1·46 (1·21, 1·77)	1·34 (1·10, 1·63)	0·004
Weight loss	416 (55)	1·44 (1·23, 1·68)	1·36 (1·15, 1·61)	1·38 (1·17, 1·64)	0·001
Dysphagia	618 (82)	1·54 (1·23, 1·92)	1·41 (1·12, 1·77)	1·35 (1·06, 1·72)	0·015
Stage					
IV *versus* I–III	100 (13)	4·14 (3·11, 5·52)	3·51 (2·60, 4·73)	2·15 (1·48, 3·14)	0·001
T+ or N+ *versus* I–III	157 (21)	2·20 (1·69, 2·87)	1·81 (1·38, 2·37)	1·11 (0·80, 1·55)	0·53
Unknown *versus* I–III	326 (43)	3·20 (2·54, 4·05)	2·08 (1·58, 2·73)	1·15 (0·80, 1·65)	0·45
Albumin					
Q2 *versus* Q1	157 (21)	0·93 (0·71, 1·20)	0·86 (0·66, 1·13)	0·91 (0·69, 1·19)	0·48
Q3 *versus* Q1	156 (21)	0·86 (0·68, 1·08)	0·81 (0·64, 1·03)	0·84 (0·66, 1·07)	0·15
Q4 *versus* Q1	157 (21)	0·64 (0·51, 0·82)	0·70 (0·54, 0·89)	0·75 (0·59, 0·97)	0·028
Unknown *versus* Q1	124 (17)	0·89 (0·69, 1·14)	0·87 (0·67, 1·13)	0·83 (0·64, 1·09)	0·17
Resection *versus* none	239 (32)	0·30 (0·25, 0·36)	—	0·43 (0·31, 0·60)	0·001

Values in parentheses are *percentages or †95 per cent confidence intervals. ‡Continuous variable; hazard ratio for increase of ten units. §Stratified by radiotherapy, chemotherapy and stent. T+, invasive tumour; N+, lymph node involvement; Q, quartile

weight loss, no reported dysphagia, higher albumin levels, stage I–III cancer, adenocarcinoma, Barrett's oesophagus and in patients who had a resection or chemotherapy or did not have a stent. There was no association with radiotherapy or with deprivation scores. Adjustment for case-mix and treatment reduced the strength of association of survival time with doctor volume, stage and resection, and eliminated the association with hospital volume. Results of the final model are shown in *Table 7*. This model included 750 patients with oesophageal cancer, 610 (81 per cent) of whom had died. The risk of death decreased by 8 per cent (hazard ratio 0·92 (95 per cent c.i. 0·85 to 0·99)), for a difference in doctor volume of ten patients per year, and there was a 29 per cent decrease (hazard ratio 0·71 (95 per cent c.i. 0·53 to 0·95)) for a volume difference of 40.

For gastric cancer, survival time was longer with larger doctor volume, younger age, no vomiting, no weight loss, no junctional tumour, stage I–III disease, higher albumin levels and in patients who had resection or chemotherapy, but was not associated with radiotherapy or deprivation scores. Adjustment for case-mix and treatment increased the strength of the association of survival time with hospital volume and eliminated the association with doctor volume. The final model included 659 patients, 534 (81 per cent) of whom had died (*Table 8*). In the full model, the hazard ratio decreased with increasing hospital volume. There was a significant interaction between hospital volume and resection ($P = 0.013$), with a hospital volume effect in the subgroup of patients who did not have a resection (hazard ratio for a difference of ten patients 0·90 (95 per cent c.i. 0·83 to 0·96)) and no effect in the subgroup who did (hazard ratio 1·00 (95 per cent c.i. 0·96 to 1·10)).

Teaching hospital status and patient deprivation scores were not independently associated with either operative mortality rate or survival time for either cancer. Sensitivity analyses recoding stage had minimal effect on hazard ratios for doctor or hospital volume. As with operative mortality, adjustment for clustering on doctor or hospital resulted in slightly lower *P* values and narrower confidence intervals for the doctor and hospital volume coefficients.

Discussion

This study confirms the diversity of case-mix, clinical practice and degrees of specialization among doctors and hospitals dealing with oesophageal and gastric cancer in a UK population of around 8 million. Overall results are similar to those found in the Welsh surgical audit[20]. In both series, more than 50 per cent of the surgeons who carried out operations for these cancers were doing less than one case per month.

Importantly, potentially curable patients are more likely to be dealt with by 'specialists' (high-volume doctors), implying that some degree of selection occurs in the process of referral. It might then be inferred that overall improvements in survival, higher rates of surgery and lower operative mortality rates achieved by high-volume doctors merely reflect this selection bias. Detailed analyses, how-

Table 8 Predictors of survival time for all patients with gastric cancer: Cox's proportional hazards models

		Hazard ratio†			
Explanatory variable	*n**	Crude	Adjusted for case-mix, and hospital and doctor volumes	Adjusted for case-mix, hospital and doctor volumes, and treatments§	Adjusted *P*§
No. of patients	659 (100)				
Doctor volume‡		0·71 (0·62, 0·82)	0·93 (0·79, 1·08)	0·98 (0·83, 1·15)	0·78
Hospital volume‡		1·01 (0·96, 1·06)	0·95 (0·90, 1·01)	0·93 (0·89, 0·98)	0·009
Age (years)‡		1·24 (1·15, 1·34)	1·17 (1·09, 1·28)	1·12 (1·21, 1·21)	0·013
Vomiting	174 (26)	1·30 (1·08, 1·56)	1·34 (1·09, 1·63)	1·28 (1·05, 1·57)	0·015
Weight loss	380 (58)	1·46 (1·24, 1·73)	1·19 (1·00, ·43)	1·21 (1·00, 1·45)	0·046
Emergency admission	387 (59)	1·65 (1·40, 1·96)	1·35 (1·12, 1·61)	1·26 (1·05, 1·52)	0·014
Junctional tumour	204 (31)	1·31 (1·10, 1·55)	1·52 (1·26, 1·83)	1·36 (1·12, 1·64)	0·002
Stage					
IV *versus* I–III	184 (28)	4·29 (3·30, 5·56)	4·18 (3·17, 5·51)	2·64 (1·89, 3·69)	0·001
T+ or N+ *versus* I–III	136 (21)	2·37 (1·80, 3·12)	2·15 (1·61, 2·87)	1·58 (1·15, 2·17)	0·005
Unknown *versus* I–III	185 (28)	3·55 (2·75, 4·58)	2·91 (2·19, 3·86)	1·62 (1·13, 2·32)	0·008
Albumin					
Q2 *versus* Q1	146 (22)	0·73 (0·57, 0·93)	0·68 (0·52, 0·89)	0·70 (0·53, 0·91)	0·007
Q3 *versus* Q1	146 (22)	0·51 (0·40, 0·65)	0·53 (0·41, 0·68)	0·51 (0·39, 0·66)	0·001
Q4 *versus* Q1	146 (22)	0·36 (0·28, 0·46)	0·44 (0·34, 0·57)	0·42 (0·32, 0·55)	0·001
Unknown *versus* Q1	73 (11)	0·57 (0·43, 0·77)	0·60 (0·43, 0·83)	0·55 (0·39, 0·77)	0·001
Resection *versus* none	255 (39)	0·31 (0·26, 0·38)	—	0·44 (0·34, 0·58)	0·001

Values in parentheses are *percentages or †95 per cent confidence intervals. ‡Continuous variable; hazard ratio for increase of ten units. §Stratified by radiotherapy and chemotherapy. T+, invasive tumour; N+, lymph node involvement; Q, quartile

ever, indicate that this is not true, as the effect remains after adjusting for age, stage and co-morbid disease.

Once case-mix is taken into account, higher-volume doctors also used a wider range of investigations. This is seemingly not due to better access to scarce facilities, as there was no association between the use of such tests and hospital volume. Not surprisingly, a greater use of diagnostic and staging investigations by high-volume doctors led to a greater proportion of patients being staged completely.

For both oesophageal and gastric cancer, the operative mortality rate decreased as the surgeons' volume increased, even after many confounding case-mix factors were taken into consideration. These are large effects: a difference in surgeon volume of ten cases per year predicts a risk reduction in operative mortality of about one-third.

Equally important was the finding of an overall survival advantage for both high-volume doctors and hospitals across the full spectrum of patients, including the majority who did not undergo surgery. Seemingly, patients thought to have a poor prognosis tend to be referred to, or remain under the care of, low-volume clinicians and hospitals. They are then less likely to be investigated and treated, and this may adversely affect their outcome. This passive management may partly explain the poor survival associated with these cancers in the UK compared with other developed countries[1]. The fact that resection rates in this and other UK studies[27,28] are lower than in North American[27,29,30] and Asian[30–32] case series would tend to support this view.

The better survival for patients with gastric cancer as a whole was associated with hospital but not doctor volume, suggesting that aspects of hospital care, such as specialized nursing, palliative care or access to resources that might influence the timing of appropriate interventions, may all play a part in improved outcome.

For patients with oesophageal cancer as a whole, improved survival was associated with doctor volume and, for both cancers, the operative mortality rate was associated with increasing doctor volume. These results imply that, for this subsite, experience of the surgical team is crucial.

In all studies of this nature, potential sources of error merit close examination. The term 'specialization' was applied here to hospitals' and doctors' patient volumes, an approach used elsewhere[4,6,13,33] and preferable to terms used in other studies such as 'teaching hospital'[34], 'multidisciplinary team'[8] or 'consultants with an interest in ...'[7]. Patient volumes have the advantage of being unambiguous and objective indicators of current practice[33].

The strengths of the study were inclusion of all acute NHS trusts in the region, large sample sizes, prospective cohort design, prospective identification of cases which allowed virtually all hospital records to be traced and examined, inclusion of a wide range of clinical information, use of a robust and objective measure of specialization, tracking of survival of almost all cases, and extensive multivariable data analyses. The main limitations were exclusion of patients not admitted to participating hospitals, reliance on hospital sources to identify cases, imperfect information on prognostic factors and variable quality of hospital records.

The study provides an overview of the great diversity of case-mix, clinical practice and degrees of specialization of doctors and hospitals in an entire region of the NHS in the UK, and identifies underlying patterns of care. It shows that more patients with potentially curable disease tend to be referred to specialist doctors. It also suggests, however, that patients of non-specialist doctors and hospitals are less likely to receive effective investigations and treatments, regardless of their clinical presentation. Viewed together with the evidence of a lower mortality rate with more specialized care of these patients, the study supports the concentration of cancer care in the UK.

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Revascularization of the superior mesenteric artery after acute thromboembolic occlusion

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Background: The outcome and prognostic factors after revascularization of acute thromboembolic occlusion of the superior mesenteric artery (SMA) are poorly documented.

Methods: Sixty patients with acute thromboembolic occlusion of the SMA had revascularization procedures at 21 hospitals from 1987 to 1998. They were registered prospectively in the Swedish Vascular Registry. Patient files were analysed retrospectively.

Results: The median age of the patients was 76 years; 73 per cent suffered from cardiac disease and 23 per cent had previous vascular surgery. Onset of symptoms was classified as sudden (30 per cent), acute (33 per cent) or insidious (37 per cent). The occlusions were thought to be either embolic (67 per cent) or thrombotic (33 per cent). The diagnosis was suspected on first examination in 32 per cent of patients, a group whose median time to operation was shorter ($P = 0.01$). Fifty-eight patients had an exploratory laparotomy and subsequent revascularization, and two were treated with thrombolysis alone. Second-look laparotomy was performed in 41, and third look in eight patients; 19 required an additional bowel resection. The overall mortality rates were 43, 52, 60 and 67 per cent at 30 days, discharge, 1 and 5 years, respectively. No patient was dependent on intravenous nutrition after 1 year. Previous vascular surgery resulted in a higher institutional mortality rate (79 per cent; $P = 0.02$). Patients who had a sudden onset of symptoms outside hospital had a better outcome (mortality rate 27 per cent; $P = 0.02$).

Conclusion: Many non-diagnostic radiological examinations were performed and a routine second-look is warranted. The results suggest that attempts at revascularization procedures for acute mesenteric ischaemia may improve the outcome.

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Introduction

Acute thromboembolic occlusion of the superior mesenteric artery (SMA) is a condition with a dismal prognosis. In a report on 53 patients who had surgery between 1969 and 1984 the mortality rate was 85 per cent[1]. Among 57 patients who had surgery between 1987 and 1993 the mortality rate was similar, 83 per cent[2]. Endean *et al.*[3] reported a somewhat lower mortality rate of 60 per cent among 43 patients treated between 1993 and 2000, 14 of whom had undergone revascularization surgery; this suggested that contemporary treatment with more frequent use of revascularization might improve the prognosis. In a review in 1995, Bradbury *et al.*[4] stated that 'data on the long-term results of emergency mesenteric revascularization are sparse and difficult to access'.

In the Swedish Vascular Registry (Swedvasc), indications for surgery, preoperative risk factors, details regarding the operative procedure, and outcome at 1 month and 1 year are reported prospectively[5]. Previously data from the registry have been used to study the incidence of, and risk factors for, bowel ischaemia after aortoiliac surgery[6,7] by combining the prospective data from the registry with retrospective data from the patient records.

The aim of this study was to review the clinical course of patients with acute thromboembolic occlusion of the SMA, and to try to identify risk factors associated with survival in the subgroup of patients who undergo revascularization.

Patients and methods

Records from Swedvasc between 1987 and 1998 were examined; only patients undergoing a vascular surgical reconstruction were registered. Among 7634 patients who had surgery for acute thromboembolism, 84 patients (1 per cent) from 26 hospitals had reconstruction of the SMA. The case notes of these patients were requested for review. Five hospitals, in which 14 patients were treated, decided not to participate. Ten of the remaining 70 patients were excluded: files could not be found for four, in three no revascularization procedure had been performed and one patient underwent reconstruction of the SMA after resection of a tumour. Two patients had surgery for aortic occlusive disease within 30 days before thromboembolic occlusion of the SMA. They were included in a cohort of patients with intestinal ischaemia complicating aortoiliac surgery, and had been studied previously[6,7]. The remaining 60 patients from 21 hospitals were included in the study.

Statistical analysis

Statistical analysis of continuous variables was performed with Levene's test for equality of variances, followed by Student's *t* test. Discrete variables were analysed with the Pearson χ^2 test. Postoperative survival was analysed according to the Kaplan–Meier method. $P < 0{\cdot}05$ was considered significant.

Results

The median age was 76 (range 35–90) years in the 35 women and 25 men studied. Preoperative risk factors included previous reconstructive vascular surgery in 14 patients, diabetes in ten, hypertension in 25, cerebrovascular disease in 14, pulmonary disease in six, renal insufficiency in four and smoking in nine patients. Forty-four patients suffered cardiac disease (*Table 1*), but only two patients were taking anticoagulant medication. Both had interruption of anticoagulation treatment under protection of low molecular weight heparin after mesenteric embolization occurred. Eight of the patients reported previous symptoms compatible with intestinal ischaemia: four described intestinal angina, two had significant weight loss and three had chronic diarrhoea.

Table 1 Characterization of cardiac disease in 60 patients with acute thromboembolic occlusion of the superior mesenteric artery

	No. of patients
Atrial fibrillation	34 (57)
Valvular disease	2 (3)
Cardiomyopathy	2 (3)
Ischaemic heart disease	15 (25)
Any kind of cardiac disease	44 (73)

Values in parentheses are percentages. Some patients had multiple pathology

Symptoms and signs

Seventeen patients (30 per cent) suffered a sudden onset of severe abdominal pain (within minutes), 19 (33 per cent) developed acute pain (within 1 h), 21 (37 per cent) had an insidious onset of pain and in three the onset was unknown. The onset was no more sudden among patients thought to have embolic disease ($P = 0{\cdot}3$).

The cause of acute occlusion was considered to be embolic in 40 patients and thrombotic in 20. The sources of emboli were atrial fibrillation (30 patients), acute myocardial infarction (five), aortic thrombus (one), cardiomyopathy (one) or unknown (three).

Diarrhoea was reported by 21 patients and was bloodstained in 11; 33 patients had vomiting. On presentation, 13 had had abdominal pain for less than 1 h, 22 for less than 2 h, 42 for less than 12 h and 15 for more than 12 h.

The signs on physical examination at presentation were recorded in 51 patients. In 27 (53 per cent) there was pain out of proportion to the signs, i.e. no sign of peritonitis in spite of intense pain. Fourteen patients (27 per cent) had signs of peritonitis. Other physical signs were distended (nine) or tympanic (four) abdomen, hyperperistalsis (two) and bloody stools on rectal examination (three). Two patients had simultaneous embolization to leg.

The diagnosis of acute SMA occlusion was suspected at the time of the first examination in 19 patients (32 per cent) because of atrial fibrillation (11), recent stroke (six) or acute embolism to the leg (two), or the history of sudden onset (eight).

Diagnosis

Patients were recorded prospectively into the Swedvasc database; analyses of laboratory results were retrospective. The median white blood cell count on admission was 16 200 (range 5000–81 000) per mm^3; only two patients had a normal count.

Plain abdominal radiography was performed in 35 patients and barium follow-through in five. Paralytic ileus was seen in eight patients, intestinal obstruction in four and in 23 patients the radiological picture was inconclusive.

Five patients were examined with duplex ultrasonography. One examination was impossible due to excessive bowel gas, in three patients arterial flow was verified in the proximal segment of the SMA and in one patient no flow was detected. Plain abdominal ultrasonography was performed in 16 patients.

Four computed tomography (CT) examinations without contrast were not diagnostic. Among six CT examinations with contrast enhancement two were diagnostic. In the false-negative examinations contrast was observed in the proximal SMA.

The diagnosis was verified by angiography in 11 and at laparotomy in 49 patients. One patient had angiography after laparotomy, followed by revascularization. Among the 12 patients examined by angiography five had a patent proximal segment of the SMA, with either a visible embolus or a distal occlusion. These lesions were interpreted as embolic. Seven patients had thrombotic occlusions at the origin of the SMA.

After investigation, a median of 16 (range 1·5–408) h after presentation, 58 patients underwent exploratory laparotomy. At the time of the operation 38 (66 per cent) were suspected to have mesenteric ischaemia. Among the 20 patients in whom the diagnosis was not suspected the preoperative diagnosis was peritonitis (nine), perforated appendicitis (five), intestinal obstruction (three), caecal volvulus, diverticulitis and gastrointestinal bleeding (one each).

The median time between presentation and operation or thrombolysis was significantly shorter among patients in whom the diagnosis was suspected at first examination (6 h) than among those in whom it was not (24 h) ($P = 0{\cdot}01$).

Treatment

All 40 patients with SMA embolism were treated by embolectomy; one had additional intraoperative thrombolysis. Among the 20 patients treated for SMA thrombosis, ten had thrombectomy. Adjunctive procedures included patch angioplasty (two), on-table angioplasty (two) and aortic thrombectomy (one). Eight patients were treated with mesenteric bypass from the aorta (seven) or the common iliac artery (one) to the SMA (five) or to both mesenteric arteries (three). Finally, two patients were treated by thrombolysis without laparotomy, one of whom also underwent angioplasty.

In all patients revascularization was considered a prerequisite for survival. Ten of 40 patients with embolic disease and 14 of 20 with thrombosis also required bowel resection at the primary procedure. A second-look operation was performed in 41 patients and a third look in eight. Nineteen patients required additional bowel resection at relaparotomy. In all, 43 patients underwent bowel resection; 42 had a resection of the small intestine (median 145 (range 10–360) cm) and 20 had a colonic resection (median 30 (range 15–120) cm).

Outcome

The 30-day and in-hospital mortality rates were 43 and 52 per cent respectively. The patients died a median of 6 (range 0–72) days after surgery. On routine follow-up the mortality rate had risen to 60 per cent at 1 year, but to only 67 per cent at 5 years.

Many of the surviving patients were dependent on intravenous nutrition during the first months. Among the 24 patients who were alive 1 year after surgery none was dependent on intravenous nutrition, but six required medication for diarrhoea.

Prognostic factors

In-hospital mortality was examined according to patient- and procedure-related factors (*Tables 2* and *3*). A history of previous vascular surgery was associated with a higher in-hospital mortality rate. Previous vascular surgical procedures were for embolism (four), chronic (four) or acute (two) ischaemia of the leg, aortic aneurysm (three) and thoracic aortic dissection. All procedures had been performed more than 1 year previously, except for two patients treated for embolism at the same admission.

Table 2 Patient-related prognostic factors after revascularization for acute mesenteric ischaemia

	No. of patients	In-hospital mortality (%)	*P*†
All patients	60 of 60	52	
Female sex	35 of 60	57	
Age > 70 years	48 of 60	50	
Age > 80 years	19 of 60	63	
Chronic bowel ischaemia	8 of 60	50	
Smoking	9 of 51	33	0·08
Diabetes	10 of 60	40	
Ischaemic heart disease	15 of 60	53	
Previous vascular surgery	14 of 60	79	0·02
Hospitalized before onset	18 of 60	50	
Embolic occlusion	40 of 60	50	
Presenting symptoms and signs			
Sudden onset of pain	17 of 57	35	0·09
Sudden onset of pain at home	15 of 57	27	0·02
Acute or sudden onset of pain (< 1 h)	36 of 57	47	
Insidious onset of pain (> 1 h)	21 of 57	62	
Presentation within 1 h*	13 of 58	62	
Presentation 1–12 h*	30 of 58	40	0·06
Presentation after 12 h*	15 of 58	67	
Vomiting as presenting symptom	33 of 60	46	
Diarrhoea as presenting symptom	21 of 60	43	
Pain out of proportion	27 of 51	41	
Peritonitis on admission	14 of 51	57	

*Time from onset of pain until first examination by physician. †Pearson χ^2 test

The median delay from the first examination by a physician to laparotomy was shorter among the patients in whom the diagnosis was suspected, either initially or after diagnostic assessment (13 h), than among those in whom the diagnosis was not suspected (24 h) ($P < 0·05$), but this did not affect outcome.

There was a non-significant ($P = 0·06$) improvement in survival in patients who presented between 1 and 12 h after the onset of symptoms. All 13 patients who were evaluated within 1 h from onset of symptoms were in hospital at the time.

Since there was a tendency to a lower mortality rate among those who had a sudden onset of symptoms, and suspecting that patients already in hospital might have an adverse outcome owing to multiple disease, these factors were combined. The 15 patients with a sudden onset of symptoms and who were not in hospital had a better outcome.

Various factors related to management were not associated with outcome (*Table 3*), including timing of bowel resection (at primary operation or at repeat laparotomy), length of bowel resected, perioperative administration of mannitol, type of thromboprophylaxis and requirement for inotropic support.

Discussion

In an 18-year retrospective review of 214 patients from Finland treated for acute intestinal ischaemia, the lowest mortality rate (51 per cent) was reported in the group of patients treated by bowel resection only, a subgroup of patients (32 per cent) with less severe disease[8]. Most patients (61 per cent) were treated conservatively by exploratory laparotomy alone, with a mortality rate of 98 per cent. Fifteen patients (7 per cent) were treated with revascularization with a mortality rate of 87 per cent. At this large regional hospital less than one revascularization procedure was performed per year. In the present multicentre review 60 patients were identified who had revascularization, with a better than expected long-term survival, and without the need for intravenous nutrition.

Table 3 Management-related prognostic factors after revascularization for acute mesenteric ischaemia

	No. of patients	In-hospital mortality (%)
All patients	60 of 60	52
Suspicion at first examination	19 of 60	53
Time to operation (h)*		
< 4	12 of 53	50
< 8	19 of 53	47
< 24	39 of 53	49
Plain abdominal radiography	35 of 60	49
Diagnosis by angiography	11 of 60	36
Diagnosis suspected before operation	38 of 58	55
Bowel resection†	43 of 58	53
Colonic resection	20 of 58	65
Second-look operation	41 of 58	51
Third-look operation	8 of 58	50
Dopamine medication	26 of 55	62
No vasoactive medication	10 of 55	40

*Time from first examination to laparotomy or thrombolysis. †Bowel resection at primary or secondary operation

Despite a high frequency of atrial fibrillation and cerebrovascular disease among the patients with embolic occlusions, only two were on anticoagulant treatment. Warfarin is underprescribed in patients with atrial fibrillation and half of those treated have subtherapeutic anticoagulation[9].

The prerequisite for successful revascularization is prompt diagnosis. The delay from the first examination to laparotomy was significantly shorter among the patients in whom the diagnosis was suspected; however, early diagnosis did not improve survival. This unexpected finding could be explained by variations in severity of the disease. Patients who were diagnosed and treated most quickly were those in hospital at the time. A higher proportion of patients who suffered a delay in diagnosis may not have been suitable for revascularization owing to extensive bowel gangrene. The present study only included patients who had a revascularization procedure.

Considering that very few patients were examined by duplex imaging or CT, it is hard to draw conclusions on the value of these examinations. That some investigations showed a preserved blood flow in the proximal segment of the SMA was explained by the fact that emboli to the SMA are often trapped where the artery tapers, some distance from its origin from the aorta.

Among 35 plain abdominal radiographs, five barium follow-throughs, 16 ultrasonographic examinations, five duplex and ten CT scans, only three resulted in a correct diagnosis. Angiography was diagnostic in 11 patients before laparotomy and two patients had local thrombolysis. Most of these diagnostic measures merely delayed revascularization.

A quick and reliable diagnostic test for intestinal ischaemia is needed to select patients for intervention. Plasma lactate concentration greater than 2·5 mmol/l is sensitive for bowel gangrene, but the test has a low specificity[10]. D-Lactate may be more specific than L-lactate[11]. The ideal laboratory test should detect ischaemia before gangrene has developed. In a pilot study a raised level of D-dimer was found to have a high sensitivity for bowel ischaemia[12]; a prospective study is under way.

More than half of the patients had abdominal pain and atrial fibrillation. However, in only 11 of the 34 patients with atrial fibrillation, and in only eight of the 15 with atrial fibrillation and a sudden onset of abdominal pain, was the diagnosis of mesenteric ischaemia suspected on first examination. A high index of clinical suspicion is fundamental.

Patients thought to have SMA thrombosis required bowel resection more frequently than those thought to have embolism. Thrombotic occlusion occurs at the origin of the SMA, flush with the aorta, and does not permit collateral flow through the first branches of the SMA.

Patients with a history of previous vascular surgery had an adverse outcome and patients with a sudden onset of abdominal pain outside hospital had a favourable outcome. It was not possible to identify any procedure-related factors affecting outcome. Although this study included a significant number of patients having revascularization, there remains the possibility of a type II statistical error.

Most Swedish surgeons performed a routine second-look laparotomy in these patients after mesenteric revascularization. The high frequency of second-look operations appeared warranted as half of the patients required additional bowel resection. The 19 patients who did not have reoperation either had no signs of peritonitis or sepsis (favourable prognosis) or a decision to withhold treatment was taken (dismal prognosis). This group had the same overall mortality rate as those who had repeat laparotomy.

In this subgroup of patients who had revascularization procedures for mesenteric ischaemia, both in-hospital and 5-year survival rates were favourable. No patient needed intravenous nutritional support 1 year after surgery. Although mesenteric ischaemia is often a diagnosis of exclusion, the large number of non-diagnostic radiological examinations may have resulted in unnecessary delay. It is important to have a high index of suspicion and to verify the diagnosis with laparotomy or angiography.

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Original article

Experimental study of the material composition of laparoscopic ports on tumour cell adherence

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Background: Laparoscopic resection of intra-abdominal malignancies has yet to be widely adopted, partly because of concerns over the possible increase in the rate of port-site metastasis. The aetiology of these is unclear, but the laparoscopic instrumentation used may influence the deposition of tumour cells at the port sites during operation. An *in vitro* model to examine tumour cell adherence to laparoscopic ports and to port sites was developed to examine this hypothesis.

Methods: A pilot study (study 1) was performed in which six smooth plastic, six ribbed plastic and six metal ports were introduced through the shaved abdominal wall of a cadaveric sheep and suspended in a water-bath containing radiolabelled LIM 1215 human colonic cancer cells for 30 min. Radioactivity on both ports and port sites was measured and the number of cells adherent to each structure was calculated. The study was expanded to include a further 36 smooth plastic ports and 36 metal ports (study 2).

Results: In study 1 metal ports were found to have significantly more adherent cells than plastic ports ($P = 0{\cdot}004$), as did ribbed ports when compared with smooth ports ($P < 0{\cdot}05$). In study 2 increased numbers of cells were again detected on metal ports ($P < 0{\cdot}001$) when compared with plastic ports. Significantly greater numbers of cells were also detected on the sites through which metal ports had passed than on sites through which plastic ports had passed ($P = 0{\cdot}03$).

Conclusion: In this model, the use of metal ports as opposed to plastic ports resulted in increased deposition of tumour cells on both ports and port sites.

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Introduction

Debate continues in the literature as to whether there is an increased risk of developing a wound metastasis within the port site following laparoscopic surgery for malignancy compared with the risk of developing a recurrence in a laparotomy wound following an open procedure for malignancy[1-3].

Many possible aetiological mechanisms have been advanced to explain this increased risk, with the majority of published work suggesting that the predominant mechanism involves the direct spread of free viable intraperitoneal tumour cells to ports and/or instruments and then to port sites during operation[4]. This would imply that some tumour cells must adhere to ports and instruments in order for them to be transmitted to port sites, which has already been shown experimentally[5].

If differences between port composition influence the ability of tumour cells to adhere to ports and hence effect contamination of the port sites, this may play a role in altering the risk of developing a port-site metastasis.

Methods

LIM 1215 human colonic cancer cells were grown in 75-ml plastic flasks containing Dulbecco's modified Eagle's medium (DMEM; ICN Biomedicals, Costa Mesa, California, USA). The cells were harvested when they were 80–90 per cent confluent across the base of the culture flasks, by washing with 20 ml phosphate-buffered saline (PBS) and then incubating them for 5 min at 37°C with 0·05 per cent trypsin solution (Gibco BRL, Gaithersburg, Maryland, USA). The free cells were suspended in 20 ml DMEM and centrifuged at 400*g* for 5 min; the supernatant was then discarded and the cells were resuspended in 20 ml PBS. An aliquot of this solution was taken and examined in a haemocytometer to calculate cell numbers, and a further small aliquot was incubated for 3 min with 0·02 per cent trypan blue solution (Flow Laboratories, Sydney, New South Wales, Australia) to calculate cell viability. Cells were radiolabelled by the addition of 1 GBq of ^{99m}Tc-labelled exametazime (Ceretec™; Amersham, Castle Hill, New South Wales, Australia) to the suspension, and then incubated for 15 min in a water-bath maintained at 37°C. To remove unbound ^{99m}Tc-labelled exametazime, cells were washed in 50 ml PBS, centrifuged at 400*g* for 5 min, and the supernatant was discarded. This process was then repeated for two more cycles before the radiolabelled cells were finally suspended in 10 ml PBS.

The radiochemical purity of ^{99m}Tc-labelled exametazime was checked before cell labelling, according to a minicolumn method[6].

Study 1

The flask containing the cell suspension was placed on a γ camera using a low-energy collimator (Serle™; Serle Nucleonics, Chicago, Illinois, USA) connected to a computer (Microdelta; Siemens, Gammasonics, Hoffmann Estates, Illinois, USA). The number of counts present in this sample was acquired over 2 min; the cells were then removed and shielded to permit measurement of background activity.

A sample of 30 million radiolabelled LIM 1215 cells was added to a water-bath that had been heated to 37°C and contained 20 litres water. An anterior abdominal wall was excised from a shaved cadaveric sheep and secured in place above the water-bath.

Three sets of six ports were placed through this abdominal wall such that the shafts were immersed in the water, in which they remained for 30 min. Circulation of the water was maintained by an agitator.

The sets of ports comprised six smooth plastic 11-mm ports (Endopath®; Johnson and Johnson Medical, North Ryde, New South Wales, Australia), six ribbed plastic 11-mm ports (Endopath®) and six metal 11-mm ports (Spartan Applied Medical, Rancho Santa Margarita, California, USA).

Each port was excised in a block containing the port site after the incubation period had ended, and the ports were then withdrawn from the port site. The radioactivity of both ports and port sites was measured individually by placing them on the γ camera for a 2-min acquisition period.

The number of cells present on each sample was calculated after correction for background activity and radioactive decay by comparing the corrected counts from each sample with the number of counts from the original sample of 30 million cells.

Study 2

In the second study a similar protocol was followed except that 36 metal ports were compared with 36 smooth plastic ports and the acquisition of data was altered to permit improved cell resolution.

A 0·1-ml sample was withdrawn from the cell suspension of 30 million radiolabelled cells in 10 ml PBS and diluted to 5 ml with 0·9 per cent saline. A further 0·1-ml aliquot of this diluted suspension was withdrawn and placed in a large-volume counter (Biosentry; AEI-EKCO, Australia) linked to a multichannel analyser (model 3100; Canberra Industries, Meriden, Connecticut, USA), with its radioactivity measured over 10 s. This provided the radioactivity from a diluted suspension containing 6000 cells. Background activity was also measured over this period.

To calculate cell numbers on ports and port sites, these were also placed separately in the large-volume counter for 10 s, and the amount of radioactivity was compared with that in the initial sample of cells, with corrections made for background activity and radioactive decay.

All measurements from the large-volume counter were repeated, and the mean value was recorded.

Statistical analysis

The data were analysed on an IBM-compatible personal computer utilizing StatsDirect® 1.612 (CamCode, Ashwell, UK). An analysis of variance (ANOVA) was performed for analysis between three groups, the Mann–Whitney *U* test for analysis between two groups, and simple linear regression for the analysis of correlation between individual port and port-site data. $P < 0.05$ was considered significant.

Cell resolution

Cell resolution was calculated as the number of cells required to produce a significant increase in activity above background levels ($P < 0.05$), and was 50 radiolabelled cells for study 1 and one radiolabelled cell for study 2.

Results

Study 1

Smooth plastic ports had the fewest tumour cells deposited on them (mean 5620 (range 711–7665)), ribbed plastic ports had more cells present (mean 7925 (range 4894–10 561)), and metal ports had the greatest number (mean 9465 (range

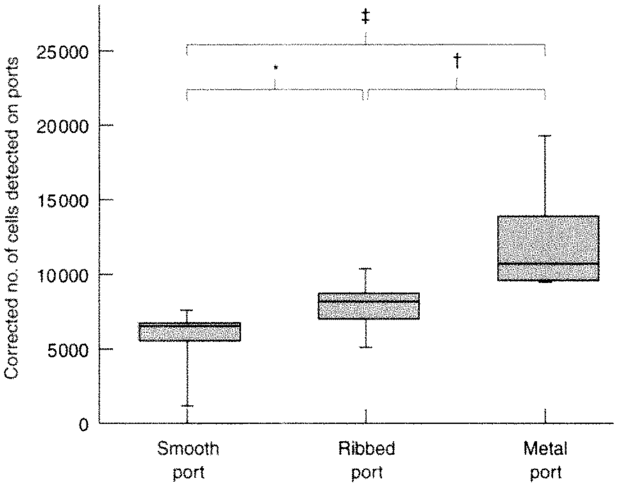

Fig. 1 Corrected number of cells found on smooth plastic, ribbed plastic and metal ports. Median, interquartile range and range are indicated by horizontal bar, shaded box and error bars respectively. *$P < 0.05$, †$P = 0.008$, ‡$P = 0.002$ (Mann–Whitney *U* test)

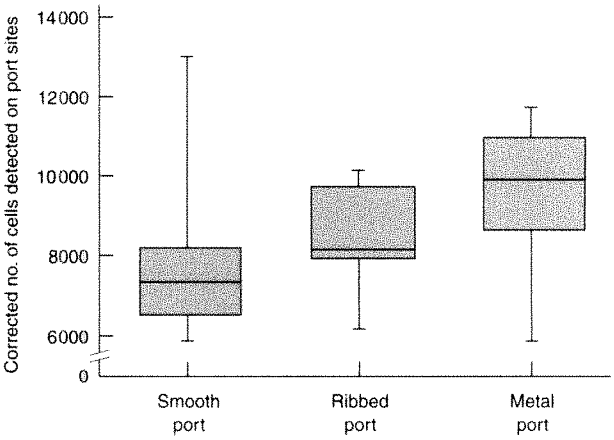

Fig. 2 Corrected number of cells found on smooth plastic, ribbed plastic and metal port sites. Median, interquartile range and range are indicated by horizontal bar, shaded box and error bars respectively. There were no significant differences between the groups

5574–11 806)). Statistically significant differences existed between these three groups of ports ($P = 0.004$).

Metal ports had significantly more adherent tumour cells than both smooth plastic ports ($P = 0.002$) and ribbed plastic ports ($P = 0.008$). Ribbed plastic ports had significantly more tumour cells than smooth plastic ports ($P < 0.05$) (*Fig. 1*).

There was no statistical difference in the number of cells deposited on the port sites between the three groups ($P = 0.55$) and, on separate analysis, no significant difference when the number of cells on metal port sites was compared with that on either smooth ($P = 0.31$) or ribbed ($P = 0.24$) ports (*Fig. 2*).

Study 2

Significantly more cells were detected on metal ports (*Fig. 3*). Additionally in this study, more cells were deposited on the port sites through which metal ports had passed, compared with the port sites through which plastic ports had passed (*Fig. 4*).

The number of cells detected on a port correlated with the number of cells on the port site through which it had been passed ($r^2 = 0.14$, $P = 0.001$).

Discussion

Previous studies have demonstrated that tumour cells adhere to instruments and ports during laparoscopic surgery[7,8] and that significantly more cells are found on the main operating port[5]. This, in conjunction with reports that operating or extraction port sites are at greatest risk of port-site metastasis[9], provides some evidence that if increased numbers of viable tumour cells are deposited on port sites this may increase the probability that at least one of these cells may successfully complete the necessary steps to form a metastasis.

Assuming that the deposition of viable tumour cells within the port site is dependent on port contamination and that this leads to the development of port-site metastasis, the overall rate of port-site metastasis should be reduced by strategies to clean or lavage the ports with cytotoxic solutions. There is some evidence that this strategy may be useful in other experimental studies: Eshraghi *et al.*[10] reported that lavage of ports with 5-fluorouracil in rats following intraperitoneal injection of tumour cells reduced the incidence of port-site metastasis from 81 to 30 per cent in comparison with controls. A similar study reported by Wu *et al.*[11] found that topical treatment of port sites and ports with povidone–iodine reduced the incidence of port-site metastasis in hamsters from 93 per cent in controls to 75 per cent.

In clinical practice, Franklin *et al.*[12] routinely lavage ports with povidone–iodine solution and have reported no port-site metastasis in a series of 191 resections.

Neuhaus *et al.*[4] have suggested that electrostatic or molecular forces may result in tumour cells adhering to ports. If this is correct, differences in the material used to construct ports might lead to additional differences in tumour cell adherence and deposition on port sites.

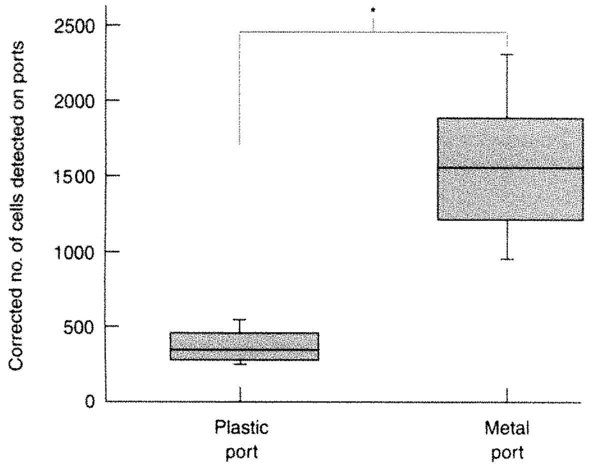

Fig. 3 Corrected number of cells found on smooth plastic and metal ports. Median, interquartile range and range are indicated by horizontal bar, shaded box and error bars respectively. *$P < 0.001$ (Mann–Whitney *U* test)

A pilot study demonstrated that metal ports had significantly more tumour cells adherent to them than ribbed or smooth plastic ports. The data from this study also demonstrated a trend for increased numbers of cells on the port site following removal of metal ports. The main study was subsequently designed to identify whether the use of metal ports in a bench-top model resulted in increased tumour cell deposition in port sites when compared with plastic ports.

A considerable difference in cell adherence between metal and plastic ports was demonstrated, and this correlated with a corresponding increase in the number of tumour cells deposited in the port site. A regression analysis, however, suggested that almost 500 cells would contaminate port sites if ports were uncontaminated. This may be due to imprecision within the study, where the zero point was not included within the 95 per cent confidence intervals and turbulence in the water-bath produced 'splashes' of tumour cells on to the underside of the abdominal wall.

The mechanism for cell adherence to ports is unclear. Earlier studies concluded that ionic and electrostatic charges are important for binding of cells to artificial surfaces[13–15]. Cellular adherence is also known to be higher to surfaces with increased irregularities[16] and less to surfaces coated with Teflon™ (Du Pont, Stevenage, UK)[17,18].

It is possible that the major factor influencing the observed differences in the present study was the variation in surface characteristics between metal and plastic ports.

However, these results may not be applicable to laparoscopic or laparoscopically assisted resections for intra-abdominal malignancies, for several reasons.

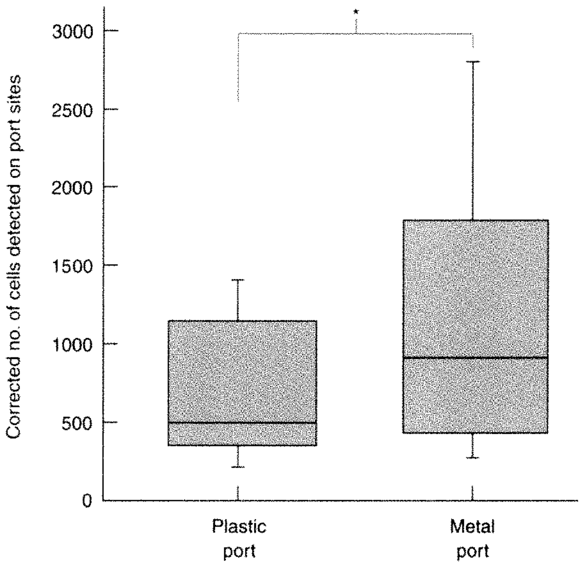

Fig. 4 Corrected number of cells found on smooth plastic and metal port sites. Median, interquartile range and range are indicated by horizontal bar, shaded box and error bars respectively. *$P = 0.03$ (Mann–Whitney *U* test)

Cell adherence varies between different cell lines and thus, although a human colorectal cell line LIM 1215 was utilized, it is possible that this cell line does not reproduce adherence patterns of most tumour cells.

One important assumption is that radioactivity measured on ports and port sites was related to ^{99m}Tc-labelled exametazime bound to cells, with all unbound ^{99m}Tc-labelled exametazime removed from the suspension by the labelling technique. In support of this, the technique is used routinely in clinical practice, with the label bound predominantly (more than 90 per cent) to cytoplasmic structures within cells, and the label leaches from cells at a rate of approximately 2 per cent per h[19]. As these studies were completed within a 3-h timeframe, such factors are likely to have had a negligible effect on the results.

Although there is circumstantial evidence that the incidence of port-site metastasis following resection of digestive tumours has decreased as experience of the technique has increased, it is still of paramount importance to reduce this rate to the lowest achievable level. Further work in a larger *in vivo* animal study is required to delineate the effect of the material composition of ports on tumour cell adherence and its possible relevance in clinical practice.

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Original article

Renal transplant fibrosis correlates with intragraft expression of tissue inhibitor of metalloproteinase messenger RNA

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Background: Chronic renal allograft nephropathy is characterized by an abnormal accumulation of extracellular matrix proteins in the glomeruli and tubulo-interstitium. The aim of this study was to determine the relationship between intragraft expression of the genes controlling the accumulation of extracellular matrix and the development of chronic renal allograft nephropathy in human renal transplants.

Methods: Forty renal allografts with stable renal function were biopsied 6 months after transplantation. Single glomeruli were plucked from the surface of these protocol biopsies and total messenger RNA (mRNA) was extracted. Reverse transcriptase–polymerase chain reaction was used to study the intragraft expression of several fibrosis-associated genes (collagen III, collagen IVα2, matrix metalloproteinase (MMP) 2, tissue inhibitors of metalloproteinases (TIMPs) 1 and 2, tenascin and transforming growth factor (TGF) β1). The level of tubulo-interstitial fibrosis was measured by quantitative immunostaining of collagen III.

Results: There were positive correlations between the level of tubulo-interstitial collagen III immunostaining and intragraft expression of the genes for TIMP-1 ($r_s = 0{\cdot}70$, $P < 0{\cdot}02$) and TIMP-2 ($r_s = 0{\cdot}59$, $P < 0{\cdot}02$). Interstitial fibrosis was also strongly correlated with the levels of TGF-β mRNA ($r_s = 0{\cdot}67$, $P < 0{\cdot}002$). Finally, TIMP-1 expression increased with TGF-β expression ($r_s = 0{\cdot}77$, $P < 0{\cdot}002$).

Conclusion: Failure of extracellular matrix degradation may be an important molecular mechanism in the pathogenesis of chronic renal allograft damage.

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Introduction

Chronic allograft nephropathy is a leading cause of late renal transplant failure, accounting for 50–80 per cent of graft losses in patients who survive after the first year[1,2]. The pathological process that underlies chronic allograft nephropathy is an excessive deposition of extracellular matrix proteins, leading to glomerulosclerosis and interstitial fibrosis[3,4]. The most abundant matrix proteins that accumulate are the type I and III fibrillar collagens and non-fibrillar type IV basement membrane collagen[5]. These proteins have been thought of as inert but, in common with other proteins, they are in a state of dynamic equilibrium, involving a balance between synthesis and degradation. Collagen degradation is controlled by a number of protease systems and derangement of these may be important in the pathogenesis of renal transplant fibrosis. The aim of the present study was to explore the relationships between intragraft expression of a number of the genes that control the accumulation of extracellular matrix and the development of chronic allograft damage in human renal transplants.

Patients and methods

Forty patients with a kidney transplant and stable renal function underwent protocol needle-core biopsy of the allograft 6 months after transplantation. All patients gave written informed consent to undergo renal transplant biopsy and the local ethics committee approved the study. Biopsies were performed under real-time ultrasonographic guidance using a spring-loaded biopsy gun[6]; two 16-G cores were obtained. The first core was processed for molecular studies of intragraft gene expression and the second was used for immunohistochemistry.

Messenger RNA extraction and quantification

The methods used have been described in detail previously[7,8]. Messenger RNA (mRNA) was extracted from isolated glomeruli by immediately immersing them into lysis buffer (Tris-HCl 100 mmol/l (pH 8), lithium chloride 500 mmol/l, ethylene diamine tetra-acetic acid 10 mmol/l, lithium dodecyl sulphate 1 per cent, dithiothreitol 5 mmol/l; Dynal, Bromborough, UK) and incubating for 1 h at 37°C with proteinase K 50 µg/ml (Roche, Lewes, UK). The total mRNA thus extracted was purified using oligo-dT-linked Dynabeads® (Dynal), which are paramagnetic beads synthesized with polythymine tails that anneal with the polyadenine tails present in all mRNA species. The glomerular mRNA was purified by applying a magnet to the side of the tube and pipetting off the supernatant. Complementary DNA (cDNA) strands were synthesized for each mRNA molecule by reverse transcription. This produced a stable Dynabead-linked cDNA library for each glomerulus. Specific cDNA molecules were amplified by polymerase chain reaction (PCR) and quantified using an enzyme-linked immunosorbent assay (ELISA) system (Thermolife Sciences, Basingstoke, UK). All primers and probes were designed in-house and are listed in *Table 1*. Where possible, primers were designed to span introns. Primers and probes were synthesized by Life Technologies or GenoSys Biotechnologies Europe (Pampisford, UK). Forward primers were synthesized 5′-biotinylated to enable capture on a biotinylated ELISA plate coated with avidin. After removal of the reverse strand, the cDNA species of interest were identified by hybridization with a specific hapten-linked probe. An antihapten antibody conjugated alkaline phosphatase was used to catalyse a colorimetric reaction in the fluid phase that was proportional to the amount of reverse transcriptase (RT)–PCR product in the ELISA well.

Competitive RT–PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also used. This glycolytic enzyme is expressed constitutively in all cells; the amount of GAPDH transcript therefore correlates with the total amount of mRNA in the glomerulus and was used to correct for differences in the cellularity of individual glomeruli, as well as differences in the efficiency of separate RT–PCR reactions. A competitor cDNA template for GAPDH was constructed and co-amplified with the unknown amount of native GAPDH. Previous investigation demonstrated a linear relationship between the ratio of competitor to native signal and the amount of competitor added for levels between 5 and 90 femtograms[8]. This relationship allowed calculation of the total amount of native GAPDH in each sample.

The expression of each gene of interest was calculated as a ratio between the amount of amplified cDNA for that gene and the amount of native GAPDH. The methods described were used to quantify the intragraft expression of glomerular collagen III, collagen IVα2, matrix metalloproteinase (MMP) 2, tissue inhibitors of metalloproteinases (TIMPs) 1 and 2, tenascin and transforming growth factor (TGF) β1.

Immunohistochemistry

Immunohistochemistry was carried out as described previously[9]. Paraffin wax sections (3 µm) were cut from renal transplant biopsies and, after dewaxing, were treated with 6 per cent hydrogen peroxide to block endogenous peroxidase. Sections were exposed to 0·1 per cent trypsin in 0·12 per cent calcium chloride, pH 7·8, for 25 min at 37°C. An indirect immunoperoxidase method was used; the primary antibody to type III collagen (Europath Diagnostics, Bude, UK) was applied overnight at 4°C. In order to facilitate subsequent image analysis a counterstain was not used.

Image analysis

Sections were viewed on a Zeiss photomicroscope (Carl Zeiss, Jena, Germany) with a video camera linked to the

Table 1 Primer and probe sequences used in this study

	5′ (forward) primer	3′ (reverse) primer	ELISA probe
GAPDH	AGAACATCAT CCCTGCCTC	GCCAAATTCG TTGTCATACC	GTTGAAGTCA GAGGAGACC
Collagen IVα2	CATGAAAGGT CTCTCTGGTG	GTATTCCGAA AAATCCAGC	CTTTGCTCCC TGGAAACCCG
Collagen III	CCTGGTACAT CTGGTCATCC	CCATTTTCAC CCTTTAATCC	ACCAGATGGA CCTATAGCAC
MMP-2	ATTGATGCGG TATACGAGGC	GGCACCCTTG AAGAAGTAGC	CTCCAGAATT TGTCTCCAGC
TIMP-1	TCCCCACACC AGAAGTCAAC	CAGGGGATGG ATAAACAGGG	GTAGTGATGT GCAAGAGTCC
TIMP-2	AACGACATTT ATGGCAACCC	ACCTGTGGTT CAGGCTCTTC	TCTATATCCT TCTCAGGCCC
Tenascin	TGAACAAAAT CACAGCCCAG	CAGTGGAACC AGTTAACGCC	TGATGGCTGA ATCTGTGTCC
TGF-β1	AAGTTAAAAG TGGAGCAGC	CACTTGCAGT GTGTTATCC	CGGTGACATC AAAAGATAAC

ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; TGF, transforming growth factor inbuilt framegrabber board of an Apple Macintosh microcomputer (Cupertino, California, USA). The histological images were imported directly to the freeware image analysis programme (NIH-image; National Institute of Health, Bethesda, Maryland, USA). Sequential fields of renal cortex were captured using the × 10 objective, moving along the central line of each biopsy specimen from one end of the available cortex to the other without overlapping. To calculate the area fraction of collagen III immunostaining, a threshold was applied to each image at a constant level. This allowed stained components rendered black to be distinguished from unstained background which was rendered white. The proportion of black to white pixels in the image was calculated and expressed as a percentage to describe the volume fraction of the tissue that was occupied by collagen III[10]. This method has been validated previously using human renal allograft biopsy material[9].

Statistical analysis

The RT–PCR data from individual glomeruli were pooled and represented as a mean value for each biopsy specimen. Complete data sets were not available for every biopsy as some of the mRNA species under consideration were not detected in all biopsy specimens. The relationship between the intragraft expression of different genes and the level of collagen III immunostaining was analysed using Spearman's rank correlation coefficient calculated by the statistical programme Graphpad® Instat (Graphpad Software, San Diego, California, USA). $P < 0\cdot05$ was considered significant.

Results

Clinical details of the 40 patients are presented in *Table 2*. Twenty-two patients were immunosuppressed with cyclosporin (15 mg per kg per day reducing by 2 mg per kg per week to a baseline of 5 mg per kg per day) and 18 with tacrolimus (0·2 mg/day) There was a positive correlation between collagen III immunostaining and intragraft expression of the mRNA for TIMP-1 ($r_S = 0\cdot70$, $P < 0\cdot02$; *Fig. 1*) and TIMP-2 ($r_S = 0\cdot59$, $P < 0\cdot02$; *Fig. 2*). Collagen III immunostaining also correlated with the level of intragraft TGF-β mRNA expression ($r_S = 0\cdot67$, $P < 0\cdot002$; *Fig. 3*). A weakly positive correlation was noted for the relationship between collagen III protein deposition and intragraft mRNA for collagen III ($r_S = 0\cdot41$, $P < 0\cdot05$). There was no correlation between collagen III protein deposition and expression of the genes collagen IVα2 ($r_S = 0\cdot16$, $P > 0\cdot2$), MMP-2 ($r_S = 0\cdot24$, $P > 0\cdot2$) or the glycoprotein tenascin ($r = 0\cdot12$, $P > 0\cdot2$). Examination of

Table 2 Patient details

Age of recipient (years)*	46(12)
Sex ratio (M : F)	23 : 17
Donor source	
Cadaveric	32
Live	8
Cold ischaemic time (h)*	16(9)
Anastomosis time (min)*	31(7)
DR mismatch*	0·6(0·6)
B mismatch*	0·9(0·7)
A mismatch*	1·0(0·8)
Delayed graft function	14
Acute rejection	13
Serum creatinine(μmol/l)*	
3 months	151(45)
6 months	137(39)

*Values are mean(s.d.)

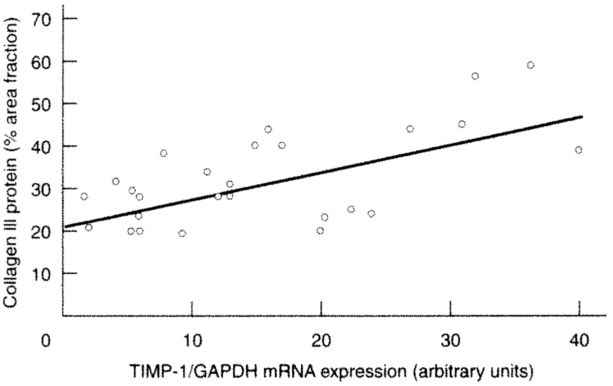

Fig. 1 Correlation between collagen III immunostaining and intragraft expression of messenger RNA (mRNA) for tissue inhibitor of metalloproteinase (TIMP) 1. GAPDH, glyceraldehyde-3-phosphate dehydrogenase

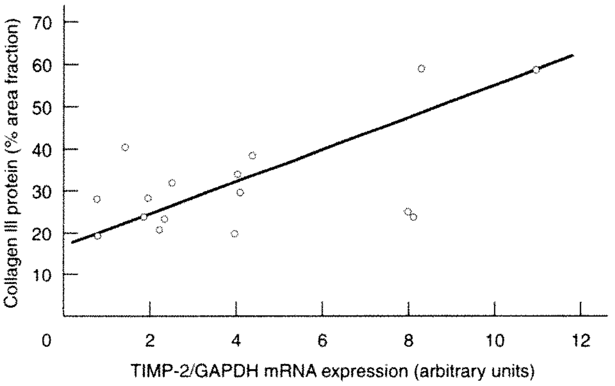

Fig. 2 Correlation between collagen III immunostaining and intragraft expression of messenger RNA (mRNA) for tissue inhibitor of metalloproteinase (TIMP) 2. GAPDH, glyceraldehyde-3-phosphate dehydrogenase

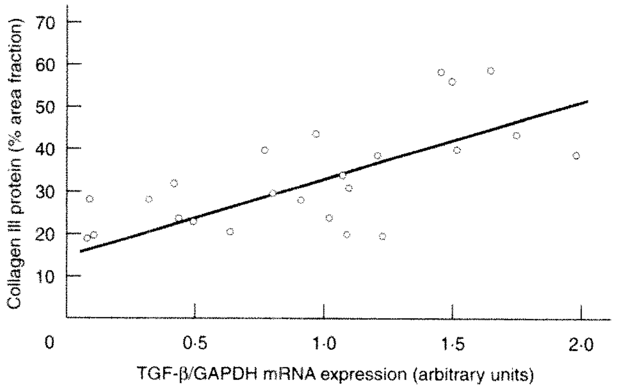

Fig. 3 Correlation between collagen III immunostaining and intragraft expression of messenger RNA (mRNA) for transforming growth factor (TGF) β. GAPDH, glyceraldehyde-3-phosphate dehydrogenase

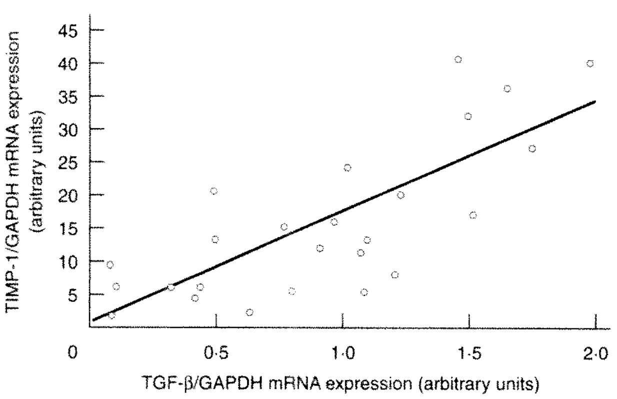

Fig. 4 Correlation between intragraft expression of messenger RNA (mRNA) for transforming growth factor (TGF) β and mRNA for tissue inhibitor of metalloproteinase (TIMP) 1. GAPDH, glyceraldehyde-3-phosphate dehydrogenase

the relationship between levels of cDNA expression for the various fibrosis-associated genes studied showed a positive correlation between TGF-β and TIMP-1 mRNA ($r_S = 0{\cdot}77$, $P < 0{\cdot}002$; *Fig. 4*).

Discussion

This study showed that intragraft expression of mRNA for TIMP-1 and TIMP-2 was significantly correlated with human renal allograft interstitial fibrosis measured by collagen III immunostaining. Previous work in the field of renal fibrosis has tended to concentrate on the role of increased synthesis of extracellular matrix proteins and the predominant theory has been that collagen deposition represents an irreversible process. It is clear, however, that in normal tissues collagens and other extracellular proteins are in a state of dynamic equilibrium in which slow synthesis of matrix is balanced by its slow removal[11]. Much of the proteolytic degradation of extracellular matrix is mediated by the MMPs[12,13], along with the plasmin-dependent pathway[14]. The extracellular activities of MMPs are regulated at several levels including the control of gene transcription (itself under the regulation of cytokines and growth factors), regulation of proenzyme activation and the inhibition of proteolytic activity by the specific inhibitors TIMP-1 and TIMP-2[12,13]. TIMP-1 and TIMP-2 both form a 1 : 1 stoichiometric complex with activated MMPs, but the exact nature by which they inhibit MMP activity has not been fully elucidated[12].

The data presented suggest that alterations in the ratio between TIMPs and MMPs in the transplant kidney may be an important molecular mechanism leading to the development of tubulo-interstitial fibrosis. The implication is that failure of the degradation of extracellular matrix, rather than simply an increase in matrix synthesis, tilts the balance towards fibrosis. As these findings were demonstrated in protocol biopsies taken 6 months after transplantation, it is probable that persistent changes in the expression of fibrosis-associated genes such as TIMP-1 and TIMP-2 may be a key feature of this response. TIMP-1 and TIMP-2 gene expression has been demonstrated in both normal and sclerotic intact human glomeruli, but the level of expression was increased threefold or fourfold in glomerulosclerosis[15,16]. Upregulation of the expression of renal TIMP-1 has also been demonstrated previously in a number of experimental models of interstitial fibrosis, including protein-overload proteinuria[17], puromycin aminonucleoside-induced nephrosis[18], hypercholesterolaemia-induced renal disease[19] and obstructive uropathy[20]. Further studies are required to measure the functional enzymatic activity of MMPs during the development of renal interstitial fibrosis in experimental models and in humans.

The use of RT–PCR allowed the measurement of gene expression in isolated glomeruli; this technique is the best available method for studying the rate of synthesis of particular proteins. Interpretation of the findings of RT–PCR data usually requires an assumption that any changes in the expression of transcripts are taken through to the protein level. In the present study a clearer picture was provided by the parallel measurement of the abundance of collagen III using quantitative immunohistochemistry by computerized histomorphometry. Previous work from this laboratory has demonstrated that the level of tubulo-interstitial fibrosis measured in this way correlated with long-term renal function. Renal transplants with less than 40 per cent collagen III deposition at 6 months after transplantation were found to have stable glomerular filtration rates over the first 2 years post-transplant, but those with more than 40 per cent collagen III demonstrated a progressive decline in renal function[9]. This method would appear therefore to be a simple and useful surrogate *ad interim* marker of chronic allograft damage.

Intragraft gene expression of the profibrotic cytokine TGF-β was found to correlate with interstitial collagen III protein deposition, confirming the findings of a preliminary analysis carried out using a smaller number of renal allograft recipients[21]. TGF-β exerts a profibrotic influence by both the stimulation of extracellular matrix deposition and inhibition of matrix breakdown by a direct downregulation of MMP activity. The finding of a positive correlation between the expression of mRNA for TGF-β and TIMPs also suggests that TGF-β has a fibrogenic action by indirectly inhibiting matrix degradation via upregulation of the TIMPs. It is accepted, however, that these data describe an association between TGF-β and TIMP expression, and this may not be a causal relationship.

This study suggests that failure of extracellular matrix degradation is an important molecular mechanism in the pathogenesis of chronic renal allograft nephropathy. The TIMPs offer a potential target for the prevention and/or treatment of progressive chronic renal allograft damage.

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Role of the neutrophil in the development of systemic inflammatory response syndrome and sepsis following abdominal aortic surgery (*Br J Surg* 2001; 88: 1583–9)

Letter 1

Sir

Although this study was well conducted, and the authors should be congratulated on their low in-hospital mortality rate of 2 per cent, I found their interpretation of the data confusing. The finding that a subpopulation of patients, shown statistically to be higher preoperative expressors of neutrophil CD16, then developed septic postoperative complications may suggest simply that they entered the operation with a septic focus. It is accepted that a recent active septic process increases the risks of postoperative sepsis and impairs phagocytic function. What evidence is there to ensure that the patients did not have clinically occult or recent sepsis on trial entry? Were leucocyte counts and inflammatory markers measured? Alternatively, are the authors suggesting that this subpopulation of patients has a genetic susceptibility to sepsis and, if so, where are the population data to support this and explain their atypically high expression of CD16? The dramatic, yet apparently statistically non-significant, difference in the operative blood loss between groups (median 800 *versus* 1500 ml) warrants explanation. Finally, the statistical test employed (Mann–Whitney *U* test) would appear fraught with potential error in view of the large number of repeated measures carried out.

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Letter 2

Sir

The role of the neutrophil in the development of sepsis or systemic inflammatory response syndrome (SIRS) following surgery is dependent on its level of activation. Markers such as β_2 integrin, CD11b and intracellular oxidative burst may be more appropriate investigative tools than the expression of a low-affinity receptor of immunoglobulin G, CD16. Furthermore, SIRS may not be associated with invading micro-organisms but rather endotoxaemia, and, as a result, different cell surface receptors may be critical in its pathogenesis. We note that phagocytosis and ingestion of *Escherichia coli* were similar for both groups prior to surgery and that the latter was reduced in the SIRS/sepsis group following surgery. In addition, CD16 expression was lower for the uncomplicated group and was unaffected by surgery. This would suggest that the capacity of the neutrophil for phagocytosis and ingestion of *E. coli* is independent of CD16 expression. On the other hand, neutrophil CD16 expression is a correlate of neutrophil apoptosis: reduced CD16 expression correlates with increased apoptosis. The authors, although recognizing a decrease in CD16 expression following stimulation with *N*-formyl-methionyl-leucyl-phenylalanine and phorbol 12-myristate 13-acetate in the sepsis/SIRS group following surgery, failed to recognize that this may be indicative of neutrophil apoptosis, a possible mechanism. Although CD16 may identify a subgroup of patients at risk of postoperative SIRS or sepsis, the mechanism involved still remains elusive.

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Author's reply

Sir

The inflammatory side of systemic inflammatory response syndrome (SIRS) has been extensively investigated by many authors including ourselves. We were interested in looking at factors predisposing to infection. CD16 presented that opportunity as it is the receptor for both opsonized bacteria and endotoxin. We recognize the role of CD16 in apoptosis, but have insufficient evidence to suggest this as a mechanism. I am investigating this, but it currently does not appear to be the explanation; hence I agree that the mechanism is still elusive. There was no evidence to suggest that these elective patients had a preoperative focus of infection or sepsis. Although the data for white cell count, C-reactive peptide and markers of neutrophil activation (CD11b, CD18 expression) were not included in the paper, these were not raised before operation.

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Cytoreduction including total gastrectomy for pseudomyxoma peritonei (*Br J Surg* 2002; 89: 208–12)

Sir

Although supporting the idea of maximal cytoreductive surgery plus heated intraoperative chemotherapy for the treatment of pseudomyxoma peritonei, the concept presented in this article cannot be supported without some critical questions. As total gastrectomy, and in particular oesophagojejunostomy are procedures with a very low morbidity rate, there is hardly any reason to perform a high-diverting jejunostomy in order to decompress the small bowel. Even in cases of small bowel paralysis a rupture of the oesophagojejunostomy is rarely seen and, if so, is usually due to poor surgical technique or impaired blood supply. In our own experience with total gastrectomy for pseudomyxoma peritonei no insufficiencies of the proximal anastomosis or prolonged small bowel paralysis was observed. From the data with missing values of the median survival, as well as the quality of life of the patients, the conclusions of this article appear very preliminary. In oncological surgery, one aim is certainly an improved long-term prognosis, but also to maintain a good quality of life. This is definitely not accomplished by a high-diverting jejunostomy and total parenteral feeding. For these reasons, it remains questionable how far we should go in the treatment of pseudomyxoma peritonei. The aim should not be to keep the patients only alive, but in life.

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Author's reply

Sir

Over past decades, patients with this condition received extensive surgical intervention, but there were no long-term survivors. With cytoreductive surgery plus intraperitoneal chemotherapy, the majority of patients show a prolonged disease-free survival of over 10 years. The requirement for the high diverting jejunostomy is controversial. In the evolution of this treatment strategy, I attempted to use duodenostomy tubes[1]. Four of the eight patients decompressed using a duodenostomy tube suffered a life-endangering complication. As a result I decided that more reliable gastrointestinal decompression was necessary.

Prolonged gastrointestinal ileus with retention of bile and pancreatic secretions within the intestine are always seen in these patients; nearly all had a total parietal peritonectomy. In my experience small bowel motility does not return for several weeks.

As we mentioned in the article, 37 of the 45 patients are currently disease-free with a median follow-up of 26 months, in a condition that is slow to progress. Ten-year follow-up is necessary in order to assess the survival benefit of treatment. This report attempted to emphasize our positive opinion regarding the safety of this approach in a difficult group of patients.

In the USA, home infusion companies exist that can provide home parenteral feeding even in the most rural parts of the country. Parenteral feeding is routinely used to move patients from hospital to home, thereby decreasing the length of hospital stay. Although this might cause considerable hardship for the patient as well as for the healthcare system in Germany, it is as simple as a phone call prior to discharge from the Washington Hospital Center. Although there is a very major impact on the quality of life of these patients while under treatment, their long-term quality of life seems to be relatively unchanged from normal and markedly improved over the preoperative status of 'jelly belly'.

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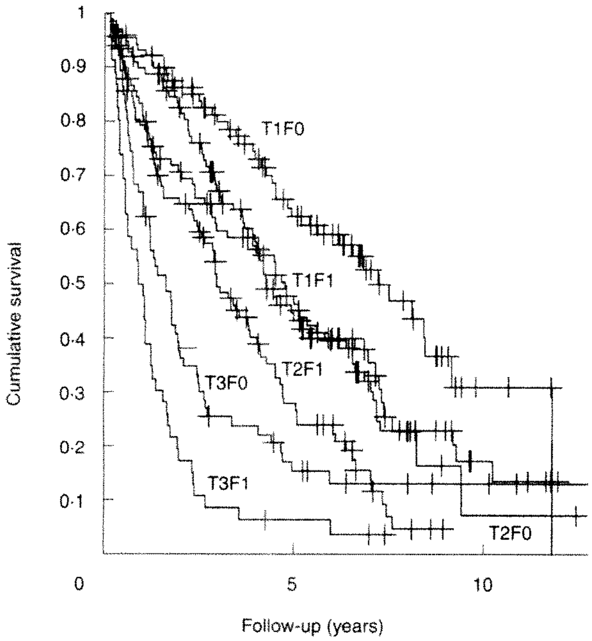
Liver fibrosis increases the risk of intrahepatic recurrence after hepatectomy for hepatocellular carcinoma (*Br J Surg* 2002; 89: 57–62)

Sir

Patients with fibrosis–cirrhosis in this study had almost a ninefold increased risk of recurrent hepatocellular carcinoma (HCC) compared to patients with no fibrosis. We believe, however, that prognosis after resection of HCC should be analysed in the context of all histopathological factors, including microscopic vascular invasion[1,2]. We recently performed a detailed pathological analysis of both tumour and underlying liver factors in 557 patients who underwent complete resection for HCC[3]. The independent predictors of death were microscopic vascular invasion, major vascular invasion, fibrosis–cirrhosis in the adjacent liver, multiple tumours and tumour size. Based on these findings a simplified T classification scheme was proposed that will be the basis for the forthcoming American Joint Committee on Cancer staging system for HCC: single tumours without vascular invasion are classified as T1; single tumours with vascular invasion or multiple tumours, none greater than 5 cm, as T2; multiple tumours, any greater than 5 cm, or tumours with major vascular invasion as T3. To account for the effect of fibrosis–cirrhosis on survival the classification allows for separate rating of T categories as either F0 (none to mild fibrosis) or F1 (moderate to severe fibrosis–cirrhosis). This modification will further refine the prognostic ability of HCC staging (*Fig. 1*).



No. at risk :

T1F0	90	74	42	21	3
T1F1	103	78	38	19	5
T2F0	96	61	31	17	1
T2F1	99	64	24	9	–
T3F0	69	25	10	6	4
T3F1	47	9	3	2	–

Fig. 1 Comparison of outcome according to stage in 557 patients who had resection of hepatocellular carcinoma. Outcome was stage-dependent ($P < 0{\cdot}001$). T, tumour; F, fibrosis classification. Figure reproduced with permission[3].

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Author's reply

Sir

We thank Drs Esnaola, Vauthey and Lauwers for their comments. We also analysed various tumour-related factors including microscopic vascular invasion (described as vascular involvement). Consistent with their study[1], vascular invasion showed prognostic significance by univariate, but not multivariate, analysis. This may be because of the limited number of patients in our series or a much higher significance of hepatitis-related factors, including fibrosis. The main point of interest in our study was the significant contribution of the occurrence of new tumours to all intrahepatic recurrence of hepatocellular carcinoma (HCC) by multicentric carcinogenesis from the underlying chronic liver disease. The study suggested that liver fibrosis represented the potential for multicentric hepatocarcinogenesis in the liver remnant. The degree of fibrosis should indeed be included in staging for HCC. Liver fibrosis is not only a significant risk factor for patient death, but also an independent predictor of intrahepatic recurrence after resection of HCC.

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1 Vauthey JN, Lauwers GY, Esnaola N, Do KA, Belghiti J, Mirza N *et al.* Simplified staging for hepatocellular carcinoma. *J Clin Oncol* 2002; **20:** 1527–36.

Review for Surgery: Scientific Principles and Practice. 3rd Ed.

L. J. Greenfield, K. D. Lillemoe, M. W. Mulholland, K. T. Oldham and G. B. Zelenock (eds)
280 × 220 mm. Pp 521 Illustrated 2002 Philadelphia, Pennsylvania Lippincott Williams and Wilkins $49 95

This is a companion volume to a main textbook, *Surgery: Scientific Principles and Practice*; a CD-ROM edition is available. It is a well written volume, divided into two sections: scientific principles (pages 1–164) and surgical practice (pages 165–521). The chapters are made up of a series of multiple choice questions (MCQs), each with four or five answers. The reader is asked to select the answers that are true. Each question is followed by a detailed explanatory comment (the correct answers are indicated) and the relevant reference to the main textbook is given. The MCQs are different in style to those found in part 1 of the UK Membership of the Royal College of Surgeons (MRCS) examination and there are no extended matching questions. An MRCS candidate would probably prefer one of the other MCQ books that are available. The 17 chapters on scientific principles are excellent and may be useful for those preparing for the viva of the MRCS part 2. However, the European reader will have to adapt to the American drug names and units. The surgical practice questions assume detailed knowledge such as that expected from a senior trainee or consultant surgeon and might be useful for those preparing for the advanced examinations.

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Chassin's Operative Strategy in General Surgery. 3rd Ed.

C. E. H. Scott-Conner (ed.)
285 × 220 mm Pp 961. Illustrated. 2002. Heidelberg: Springer. £125 50

This third edition is published 8 years after the second. Much is the same but there are eight new chapters describing procedures that have appeared in the meantime. There are also new authors of the 'concepts' chapters, all of whom are from America. This strengthens the impression that the book is meant primarily for the domestic readership. It is good that operations that nowadays are uncommon are still included, as conceptually and strategically they often form the basis of the tactics of laparoscopic procedures. The drawings and text in chapter 2 on the importance of a surgeon's foot position seem superfluous to me. Similarly, the appendix showing photographs of instruments used during open and laparoscopic surgery might as well be omitted. The text is, for the most part, easy to read and the illustrations of the different operations are clear. A strong point of the book is that it includes discussion on the conceptual basis of a strategy to help the surgeon avoid common pitfalls and the operative techniques in a logical order. This is, however, also true of other surgical atlases on the market. If I bought this one it would be because of the quality of the illustrations.

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Scientific Surgery

The *BJS* is committed to the practice of surgery based on scientific evidence. Each month we will publish a list of randomized trials and meta-analyses collated from English-language publications. A collection of all these papers will be available in the Scientific Surgery archive which will be held on the *BJS* website together with links to free Medline sites where the full papers can be accessed (see below for details). If you have recently published a randomized trial that has not been featured in Scientific Surgery, the Editors would be pleased to receive a reprint and consider its inclusion.

Moller AM, Villebro N, Pedersen T, Tonnesen H. Effect of preoperative smoking intervention on postoperative complications: a randomized clinical trial. *Lancet* 2002; **359:** 114–17.

Formal counselling 6–8 weeks before surgery reduced the overall complication rate from 52 to 18 per cent ($P = 0.0003$) in this trial which included 120 patients. The biggest reduction was in wound-related complications (31 *versus* 5 per cent, $P = 0.001$).

O'Boyle CJ, Watson DI, Jamieson GG, Myers JC, Game PA, Devitt PG. Division of short gastric vessels at laparoscopic Nissen fundoplication. A prospective double-blind randomized trial with 5-year follow-up. *Ann Surg* 2002; **235:** 165–70.

Some 102 patients were evaluated 5 years after surgery. There was no significant difference in reflux symptoms or dysphagia scores, but patients who had short gastric vessel division had an increased incidence of bloating.

Vrijland WW, Tseng LNL, Eijkman HJM, Hop WCJ, Jakimowicz JJ, Leguit P *et al.* Fewer intraperitoneal adhesions with use of hyaluronic acid–carboxymethylcellulose membrane. A randomized clinical trial. *Ann Surg* 2002; **235:** 193–9.

Seventy-one patients who had a Hartmann's procedure were randomized. At reoperation (42 patients), although the incidence of adhesions was similar, their severity was reduced by the membrane.

Zhang H, Chen J, Kaiser GM, Mapudengo O, Zhang J, Exton MS *et al.* The value of partial splenic autotransplantation in patients with portal hypertension. A prospective randomized study. *Arch Surg* 2002; **137:** 89–93.

Twenty patients were included; ten had some splenic tissue reimplanted in the retroperitoneal space. There was evidence that this preserved immune function: serum tuftsin and immunoglobulin M levels were maintained.

Renehan AG, Egger M, Saunders MP, O'Dwyer ST. Impact on survival of intensive follow up after curative resection for colorectal cancer: systematic review and meta-analysis of randomized trials. *BMJ* 2002; **324:** 813–16.

Five trials which included 1342 participants were reviewed. Intensive follow-up reduced all-cause mortality (combined risk ratio 0·81, 95 per cent confidence interval 0·7 to 0·94). The effects were most pronounced in studies that used computed tomography and serial carcinoembryonic antigen assays.

Bajetta E, Procopio G, Ferrari L, Martinetti A, Zilembo N, Catena L *et al.* A randomized, multicentre prospective trial assessing long-acting release octreotide pamoate plus tamoxifen as a first line therapy for advanced breast carcinoma. *Cancer* 2002; **94:** 299–304.

Some 199 patients with recurrent or metastatic breast cancer were included; all were oestrogen receptor negative and patients who had chemotherapy were excluded. The addition of octreotide did not affect outcome (response rate 20·2 per cent *versus* 21 per cent for tamoxifen alone) and the trial was stopped.

Weeks JC, Nelson H, Gelber S, Sargent D, Schroeder G for the Clinical Outcomes of Surgical Therapy (COST) Study Group. Short-term quality-of-life outcomes following laparoscopic-assisted colectomy *versus* open colectomy for colon cancer. A randomized trial. *JAMA* 2002; **287:** 321–8.

A total of 429 patients participated in this study; analgesia requirement was reduced and there were short-term quality-of-life advantages for patients who had laparoscopic surgery.

Chan FKL, To KF, Wu JCY, Yung MY, Leung WK, Kwok T *et al.* Eradication of *Helicobacter pylori* and risk of peptic ulcers in patients starting long-term treatment with non-steroidal anti-inflammatory drugs: a randomized trial. *Lancet* 2002; **359:** 9–13.

One hundred patients due to start non-steroidal anti-inflammatory drugs were tested for *Helicobacter pylori*; those positive were randomized to eradication or control groups. At endoscopy 6 months later, eradication therapy reduced the ulcer rate from 34 to 12 per cent ($P = 0.0085$).

McColl KEL, Murray LS, Gillen D, Walker A, Wirz A, Fletcher J *et al.* Randomized trial of endoscopy with testing for *Helicobacter pylori* compared with non-invasive *H. pylori* testing alone in the management of dyspepsia. *BMJ* 2002; **324:** 999–1002.

Patients referred for investigation of dyspepsia were included. In 586 patients reviewed after 12 months, dyspepsia scores were similar. Only 8·2 per cent of patients randomized to breath testing alone required endoscopy and no significant pathology was missed in the control group.

Mikuni N, Oya M, Komatsu J, Yamana T. A prospective randomized comparison between an open haemorrhoidectomy and a semiclosed (semiopen) haemorrhoidectomy. *Surg Today* 2002; **32:** 40–7.

Thirty-four patients were studied. Clinical results according to anorectal physiology studies after operation were similar but pain scores 1 week after surgery were higher after the semi-closed procedure.

The *BJS* website can be found at *www.bjs.co.uk*. In addition to details of current and previous articles featured in Scientific Surgery, the website contains details of the Editorial Team and Board, instructions to prospective authors, links to other sites (such as The Cochrane Library) and forthcoming surgical events. The Editors welcome comments and suggestions about the website, either in writing or through the website address.

Instructions to authors

BJS publishes original articles, reviews and leading articles all of which are submitted to peer review.

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The Editorial Board of the *BJS* encourages submission of review articles or meta-analyses on topics of interest. Any topic will be considered, but priority will be given to those addressing a major current problem. The author of each accepted article will receive an honorarium. Before submission of a meta-analysis of randomized trials, authors are encouraged to consult the QUORUM statement, designed to assist in improving the quality of the submission (*Lancet* 1999, 354: 1896–900). Potential authors are advised to contact: Mr J. A. Murie, University Department of Surgery, The Royal Infirmary, Edinburgh EH3 9YW, UK.

Leading articles

The Editors commission leading articles that are 600–900 words in length and address controversial topics of current interest. They should be supported by no more than ten key references. Submissions may be subjected to external review and assessment by the Editorial Board before acceptance. The Editors retain the right to alter style and shorten material for publication. An honorarium is payable on publication.

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The Editors welcome topical correspondence from readers relating to articles published in the Journal. Responses can be sent

electronically via the *BJS* website (www.bjs.co.uk) or by post. All letters will be reviewed and, if approved, appear on the website. A selection of these will be edited and published in the Journal. Letters must be no more than 250 words in length. Letters submitted by post should be typed on A4-sized paper in double spacing and should be accompanied by a disk.

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For detailed guidance on the handling of statistical material consult *Br J Surg* 1991; **78**: 782–4. In evaluating a manuscript the Editors and statistical referees will consider the design of the study, the presentation and analysis of data and the interpretation of results.

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Set out clearly the objectives of the study, identify the primary and secondary hypotheses, the chosen end-points and justify the sample size. Investigators embarking on randomized controlled studies may wish to consider the CONSORT statement (www.consort-statement.org).

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Whenever possible use graphical presentation to illustrate the main findings of a study. The use of standard deviation and standard error should be clearly distinguished and presented in parentheses after the mean values.

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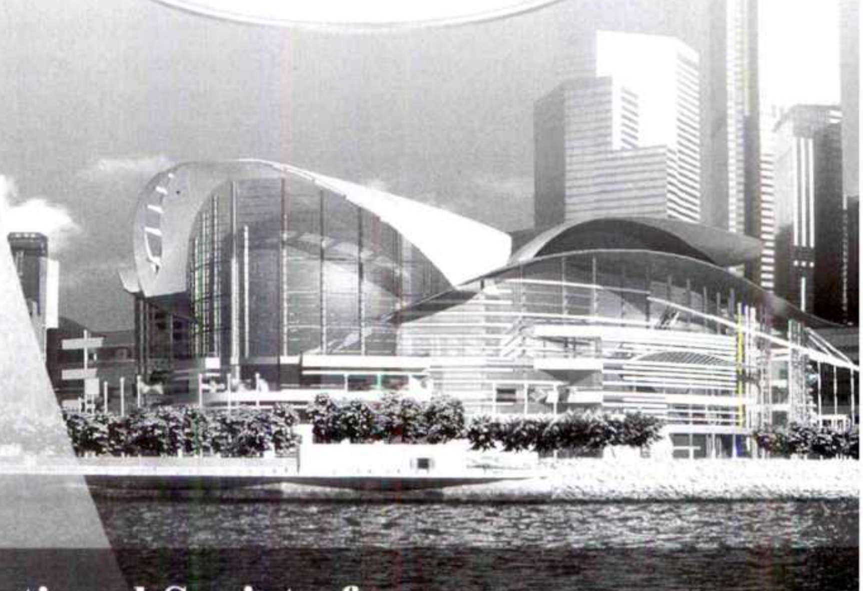

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Two journals in a joint venture

A short history of the partners. In 1919 a Swedish surgeon, Einar Key, transformed the *Archives of Scandinavian Medicine*, founded in 1869, into *Acta Chirurgica Scandinavica*, with the aim of forming a journal for Scandinavian surgeons. The journal accepted articles in English, French and German, according to the wishes of the author. In 1991, to reflect the increasingly international character of the publication, *Acta Chirurgica Scandinavica* changed its name to the *European Journal of Surgery* (*EJS*), with the aim of serving European surgeons and colleagues further afield.

The *British Journal of Surgery* (*BJS*) was founded by Ernest William Hey Groves and Berkeley George Andrew Moynihan (Lord Moynihan) in 1913, the first issue appearing in July of that year. Financial support came from Hartland Wright, a Bristol publisher (the first publisher of *BJS*). A profit-sharing agreement between the Editorial Committee and the publisher ended in 1983 when the latter's share was bought out; since then ownership of *BJS* has rested with The British Journal of Surgery Society Limited, a registered charity. *BJS* has had the good fortune to be a successful publication since its inception and is now recognized as one of the top international surgical journals.

Just as clinical surgery is now changing faster than ever before, with rapid evolution of biological knowledge and advancement of technology, so is scientific journal publishing changing ever more quickly. No longer is it sufficient to publish solely on paper. Electronic publishing is now essential, as is the ability to provide an interactive website. Within journal offices, manuscripts must be handled ever more efficiently in a competitive world.

It was with this background that the owners of *BJS* and *EJS* met two years ago to discuss the possibility of combining the strengths of their individual journals in a single publication. Since then there has been a continued dialogue aimed at providing a strong and important forum for surgical knowledge and clinical development, not only for Britain and Europe, but also for the international community. The fusion of the publications is a natural development as the parent journals have common objectives, and their backgrounds and traditions are similar. The owners and editors look forward to this stimulating joint venture. The first issue of *BJS* incorporating *EJS* will appear in January 2003.

J. A. Murie
Editor-in-Chief, the *British Journal of Surgery*
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Leading article

Adjuvant therapy for rectal cancer cannot be based on the results of other surgeons

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Worldwide, colorectal cancer is either the commonest or the second commonest cause of cancer death, and the overall 5-year survival rate is only about 40 per cent. In the light of this, perioperative adjuvant chemoradiotherapy has been widely advocated as necessary to increase disease-free survival and to decrease local recurrence. A meta-analysis of adjuvant chemotherapy has shown a 9 per cent increase in the survival rate for patients with rectal cancer, compared with surgery alone[1]. Similarly, adjuvant radiotherapy (both preoperative and postoperative) has been shown to reduce the local recurrence rate by up to 50 per cent.

However, localized disease that can be completely resected may not need chemotherapy because the aim of adjuvant chemotherapy is to destroy occult metastases and promote the chance of survival. The problem in this situation is the recognition of patients who harbour occult metastases despite an apparently curative resection. Although operative and pathological staging may point to surgery as being curative, there is still the chance of distant recurrence. Distant recurrence in a patient staged as not having distant disease at the time of operation is related mainly to the insensitivity of current staging methods in detecting occult metastatic lesions. In a nutshell, distant recurrence may be described as a failure of accurate staging. Hence potentially curable patients with features that point to a high risk of distant recurrence should be given chemotherapy[1].

Local recurrence is another matter altogether. Surgical technique directly influences the local recurrence rate in potentially curable patients, and local recurrence may therefore be regarded largely as a failure of technique. In this context it is interesting that technical training, even of established surgeons, improves technical expertise and leads to decreased rates of local recurrence[2,3]. It is also relevant that such technical expertise may not be obtained solely through the number of operations performed[4,5]. In view of the lack of any accreditation or certification for many surgeons professing to be trained colorectal specialists, it is hardly surprising that local recurrence, even for potentially curative Dukes' A–C lesions, varies widely, from 2·6 to 32·0 per cent[3,6,7].

This wide variability between surgeons hints at inherent differences in the technical art of surgery[4,8]. The science of surgery may be learned and so effectively logged by attendance at conferences, audit or even examination[5,9,10]. However, even if the craft of surgery is well demonstrated, not all observers will become as skilled as the 'master craftsman'. This is where the big difference lies: surgical craftsmanship, like most art forms, may be honed under a 'master craftsman', but only the talented apprentice will go on to produce consistently outstanding results.

In the light of this, adjuvant radiotherapy cannot be withheld from any patient just because the surgeon practises total mesorectal excision (TME), which has been reported in association with a local recurrence rate of 2·6 per cent. A particular surgeon may be a poor craftsperson, who cannot recognize tissue planes and who may produce a very high local recurrence rate. Conversely, adjuvant radiotherapy cannot be imposed on a patient just because clinical trials have shown that local recurrence is reduced dramatically with radiotherapy. A gifted surgeon with a very low local recurrence rate may not reap the same benefit from radiotherapy after rectal surgery as his or her less skilled colleague after poor technical surgery.

An obvious advantage of TME, with its associated very low local recurrence rate, is that its masterly usage may allow a surgeon to do away with routine perioperative radiotherapy and its attendant complications. In this context it is interesting that two papers[5,10] in this issue of *BJS* advocate the combination of TME with radiotherapy and describe low rates of recurrence. However, such a combination does not prove that the surgeon has performed a good technical job; these results may simply mean that recurrence rates have been kept low by the addition of radiotherapy after TME. To espouse the benefits of combining TME and radiotherapy[5,10] as a routine would erase the benefit of an excellent operation performed by a master surgeon by needlessly exposing patients to the complications of radiation. On the other hand, surgeons with frequent local recurrence after TME will still see great benefit from radiotherapy. The time is ripe for colorectal surgeons to audit their individual results for comparable cases after TME with, and without, radiotherapy.

A major recent meta-analysis of 22 randomized trials of adjuvant radiotherapy for rectal cancer considered 8507 patients and concluded that radiotherapy had neither survival benefit (62 per cent) nor improvement in resectability rate (85 per cent) over surgery alone (63 and 86 per cent respectively)[9]. Furthermore, the literature on the efficacy of chemoradiotherapy is conflicting[11]; there is hardly any evidence that chemoradiotherapy is superior to radiotherapy alone for curable rectal cancer. Poor results are often blamed on patient selection or poor study design[11].

The truth is that each surgeon must audit his or her own results, and medical and radiation oncologists must know these results before they can advise their patients appropriately. Surgical patients, unlike purely medical patients, cannot be properly counselled for or against adjuvant therapy based solely on the results of 'that particular trial which showed a significant result'. This is an important matter that is rarely considered; it is not discussed in the most recent papers advocating chemoradiotherapy for rectal cancer[5,10,12].

A surgeon with an unacceptably high rate of local recurrence in potentially curable patients may do one of three things. First, he or she may try to upgrade technical skill under the guidance of a 'master' surgeon. Second, such a surgeon may add routine chemoradiotherapy to decrease the local recurrence rate. Finally (perhaps the best and most difficult option) the surgeon may refer patients to a colleague with better results. A more fundamental problem, of course, is recognition at the training stage of those who should, and those who should not, develop a career in technically demanding surgery.

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Leading article

Differences in clinical performance

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Clinical governance, publication of league tables in national newspapers and, in the UK, the Bristol Inquiry have fundamentally changed performance management in healthcare. There is now pressure to publish individual surgeon's results. If these initiatives are to deliver better healthcare they must first overcome fundamental limitations in traditional approaches to analysing performance variation. If they do not, performance management itself will become an obstacle in the search for improvement.

Table 1 shows the results of a study of surgeon-specific mortality rates after colorectal cancer surgery[1]. Ranking the mortality rates or the adjusted hazard ratios, with or without statistical tests, invites the interpretation that some surgeons are better than others. Furthermore, since a hazard ratio of 1 is defined as neutral, surgeons with a hazard ratio above 1 may be considered a hazard to their patients. The basic premise in this kind of analysis is that variation is classified as acceptable or unacceptable. It is a short step from this to the authors' conclusions 'Some surgeons perform less than optimal surgery; some are less competent technically than their colleagues...'. To improve surgical outcomes the next logical step is to act on the unacceptable surgeons. This is precisely what the study recommends. Less competent surgeons should stop operating; work should be concentrated in the hands of the more competent. The recommendations are entirely consistent with the interpretation, and the interpretation is consistent with traditional approaches to data analysis. Surprisingly, there is another way to look at these data.

Table 1 Mortality rates after colorectal cancer surgery

Surgeon	Total	Died	Case-mix-adjusted hazard ratio
A	98	16 (16)	1·10
B	66	8 (12)	1·03
C	58	9 (16)	0·87
D	52	7 (13)	1·09
E	52	15 (29)	1·09
F	46	5 (11)	0·86
G	38	3 (8)	0·86
H	37	11 (30)	1·61
I	36	5 (14)	0·91
J	34	7 (21)	1·05
K	32	4 (13)	0·59
L	21	2 (10)	0·97
M	21	3 (14)	0·79

Values in parentheses are percentages

In the early part of the 20th century, a physicist called Walter A. Shewhart was charged with improving the quality of telephones in Bell Laboratories, USA. His work there won him the accolade 'Father of modern quality control'[2,3]. Shewhart's ideas can be easily illustrated. Six signatures are shown in *Fig. 1*. Consider the five 'BJS' signatures on the left. Two important observations can be made. First, despite being produced under the same conditions and by the same process, they show variation. Second, although they show variation, they are all recognizably the same signature. The variation is controlled: it lies within certain limits. If nothing is known about the underlying process, one would be justified in suggesting that the process appears to be stable.

What would traditional approaches to data analysis tell us about these signatures? The five signatures could be compared to a standard, and some would fall below the standard. A league table could be created, ranking the signatures from best to worst. A statistical test might identify one signature as significantly different from the others. These approaches invite punishment of the writer of the worst signature and reward for the writer of the best signature. Yet from the point of view of the underlying process, the five signatures are identical. No signature is better or worse than the others. If the desire is to improve these signatures, a change is needed in the way all signatures are written, not just the ones that fail an arbitrary test.

Now consider the sixth signature. It is clearly different from the others. A casual look suggests that this variation must have a special cause. If this kind of variation is to be addressed, the need is to identify this special cause and prevent it from interacting with an otherwise stable process. In this case, the sixth signature was produced under the same conditions as for the other five, but with the writer using his left hand. By finding this out a simple lesson is learned: one hand is better than the other at writing signatures.

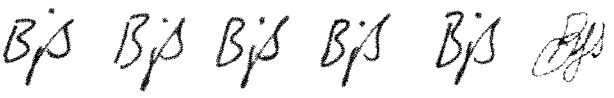

Fig. 1 Signatures

Shewhart's theory of variation categorizes variation according to the action needed to reduce it. To reduce common cause variation one must act on the process. To reduce special cause variation one must find and act on the special cause(s). To help distinguish between these two kinds of variation Shewhart devised control charts (*Fig. 2*). These charts have three lines. The central line is the mean or median, and the upper and lower lines are termed control limits. Data points outside the control limits (or unusual data patterns) indicate a special cause. Control charts are a guide towards the most appropriate action. Like any diagnostic tool, they are not infallible. Sometimes an outcome resulting from common cause variation is mistakenly taken to indicate a special cause. Sometimes an outcome resulting from a special cause is mistakenly attributed to common cause variation. It is impossible to reduce the frequency of both errors to zero. In the light of this Shewhart chose to set the control limits at a level that minimized the economic consequences of both kinds of mistakes. To do this he set the control limits three sigma from the mean (or median). This choice was based on a combination of mathematical theory, empirical evidence and pragmatism.

If the data in *Table 1* are observed with the aid of a control chart (*Fig. 2*), profoundly different conclusions are reached to those of the original study. All but one of the surgeons' results are consistent with common cause variation. The appropriate action to improve their surgical mortality rates is to change fundamentally the underlying process of colorectal cancer care. For example, screening for colorectal cancer might be introduced or steps taken to reduce the proportion of cases presenting as an emergency. In contrast, surgeon H's mortality rate is consistent with special cause variation. This should be investigated, the special causes identified and, if possible, eliminated. One should check the data systematically, the case-mix, the processes of care (from index referral to care before and after surgery), the surgery and, finally, the surgeon. In this way one will learn how to improve surgeon H's results. So reviewing the data in *Table 1* with the aid of Shewhart's theory of variation directs us away from labelling surgeons as incompetent or otherwise; instead one is asked to consider what actions are required to deliver improvement.

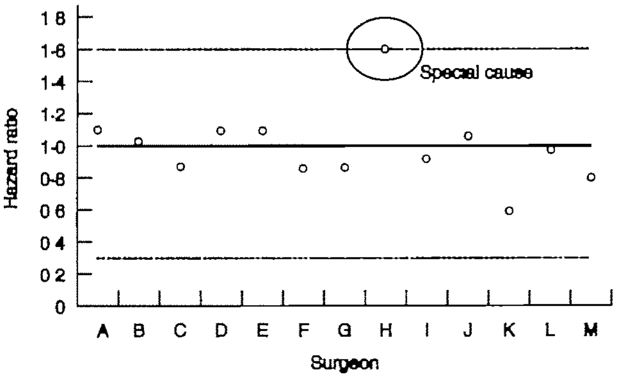

Fig. 2 Control chart. The solid line represents the median value and dotted lines denote upper and lower control limits

League tables, standards, and red and green traffic lights emphasize differences in performance. Intuitively one knows that sometimes these differences tell us nothing. Faced with so many influences on surgical outcome, control charts unlock the information contained in variation. By distinguishing between common and special cause variation they advise on when one is likely to learn from investigating differences and when one is not. They are a guide to action, a vital link between performance monitoring and improvement. They clearly work with health service data[4]. They offer a way forward.

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Review

Botulinum neurotoxin and other treatments for fissure-*in-ano* and pelvic floor disorders

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Background: The management of disorders of the lower gastrointestinal tract, such as chronic anal fissure and pelvic floor dysfunction, has undergone re-evaluation recently. To a large extent this is due to the advent of neurochemical treatments, such as botulinum neurotoxin injections and topical nitrate ointment.

Methods and results: This review presents, *inter alia*, current data on the use of botulinum neurotoxin to treat lower gastrointestinal tract diseases, such as chronic anal fissure for which it promotes healing and symptom relief in up to 70 per cent of cases. This agent has also been used selectively to weaken the external anal sphincter and puborectalis muscle in constipation and in Parkinson's disease. Symptomatic improvement can also be induced in anterior rectocele by botulinum neurotoxin injections.

Conclusion: Botulinum neurotoxin appears to be a safe therapy for anal fissure. It is more efficacious than nitrate application and does not require patient compliance to complete treatment. While it may also be a promising approach for the treatment of chronic constipation due to pelvic floor dysfunction, further investigation of its efficacy and safety in this role is needed before general usage can be advocated.

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Introduction

Since the discovery that botulinum neurotoxins inhibit neuromuscular transmission[1], these powerful poisons have been used as drugs with many indications. They were first used to treat strabismus[2], and local injections of botulinum neurotoxin are now considered a safe and efficacious treatment for both neurological and non-neurological conditions[3]. One of the most recent applications in this field is as a treatment for disorders of the gastrointestinal tract. Botulinum neurotoxin injections are not only potent in blocking skeletal neuromuscular transmission, but also block cholinergic nerve endings in the autonomic nervous system[4–6]. The capability to inhibit contraction of gut smooth muscle was first suggested by *in vitro* observations and later demonstrated *in vivo*[7]; it has also been shown that botulinum neurotoxin does not block non-adrenergic non-cholinergic (NANC) responses mediated by nitric oxide. This has further promoted interest in using the neurotoxin as a treatment for overactive smooth muscles and sphincters, such as the anal sphincter in the treatment of anal fissure, and the puborectalis muscle in the treatment of outlet-type constipation and anterior rectocele.

Anatomy and physiology of the pelvic floor

The complex anatomy and physiology of the anal canal and rectum, responsible for maintaining continence, are susceptible to disease[8–10]. There is an intrinsic relationship between the anal canal, the rectum and pelvic floor musculature. The main component of the pelvic floor is the levator ani muscle; puborectalis muscle represents the most medial portion of the levator ani muscle and is situated immediately cephalad to the deep component of the external anal sphincter (EAS) (*Fig. 1*). Puborectalis is a U-shaped strong loop of striated muscle that pulls the anorectal junction to the back of the pubis and forms the anorectal angle.

The anal sphincter complex consists of two overlapping sphincters[8–11]. The EAS is composed of striated skeletal muscle. The internal anal sphincter (IAS) is the involuntary, smooth muscle component of the sphincter complex. Being in a state of continuous maximal contraction, the IAS is a natural barrier to the involuntary loss of stool and gas, and is responsible for 50–85 per cent of resting anal tone. This relies on a combination of intrinsic myogenic and extrinsic autonomic neurogenic properties[11,12]. Being of visceral

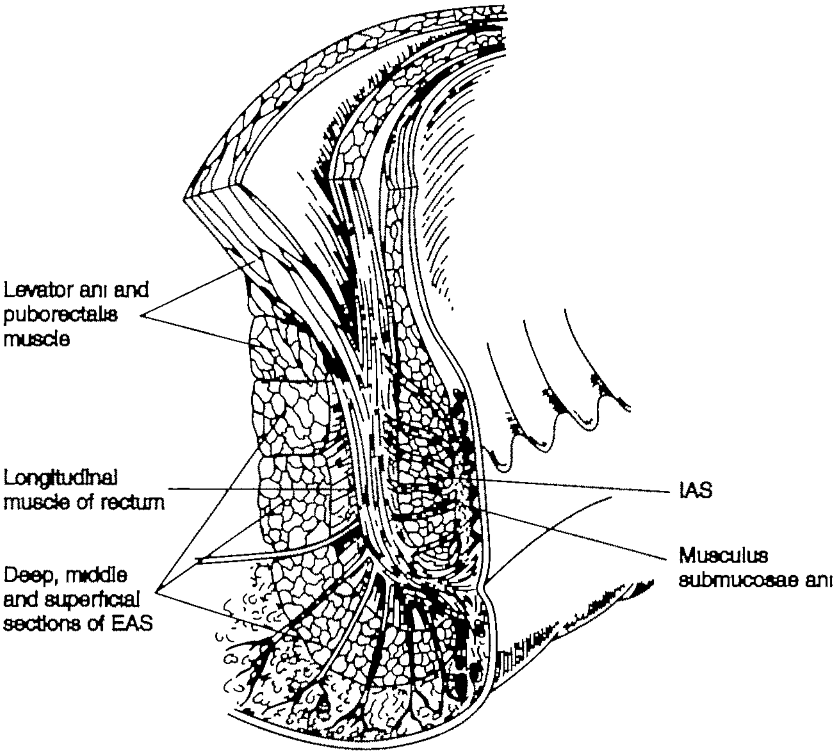
Levator ani and puborectalis muscle
Longitudinal muscle of rectum
Deep, middle and superficial sections of EAS
IAS
Musculus submucosae ani

Fig. 1 View of the levator ani muscle and the anal sphincters. EAS, external anal sphincter; IAS, internal anal sphincter

origin, the IAS is supplied by both sympathetic and parasympathetic nerves; in addition, the enteric nervous system modulates its tonic activity. Recently, it has been shown that the longitudinal layer and the circular smooth muscle receive an intrinsic nitric oxide-inhibitory innervation[13,14], and the carbon monoxide and haem oxygenase pathway may play a role in neurally mediated IAS relaxation[15].

In vitro studies on strips of human IAS have revealed excitatory α-adrenergic receptors, inhibitory β-adrenergic receptors, excitatory or biphasic cholinergic receptors, and inhibitory NANC receptors. Noradrenaline induces contraction of isolated IAS strips, while adrenaline produces a variable response and isoprenaline induces relaxation[16,17]. Blockade of α_1-adrenoceptors by a single oral dose of indoramin causes a long-lasting reduction in resting pressure along the length of the anal canal[18].

The mechanisms responsible for both faecal continence and defaecation are complex and interrelated[12]. To achieve defaecation, descending commands from the brain are transmitted to the sacral parasympathetic neurones that initiate a motor programme in the colon. The enteric nervous system in the colon is responsible for coordinating contractions of the smooth muscles and some striated pelvic floor muscles. Motor neurones located in the anterior column of the sacral spinal cord innervate, via the pudendal nerve, the EAS and the pelvic floor musculature. Tonic discharges of these neurones cause contraction of the EAS and the pelvic floor musculature, which maintain continence[12]. Inhibition of such tonic discharge is necessary for defaecation. A faecal bolus in the rectum results in reflex relaxation of the IAS, the so-called rectoanal inhibitory reflex (RAIR). There is agreement on the local intramural nature of the reflex, and morphological data provide compelling anatomical evidence that nitric oxide mediates RAIR[11,12,16,17,19].

Botulinum neurotoxins

The clostridial neurotoxin family is composed of tetanus neurotoxin and seven serotypes of botulinum neurotoxin, designated as serotypes A–G[20]. Neurotoxin produced under anaerobic conditions by *Clostridium botulinum* exerts its paralytic effects at the neuromuscular junction by inhibiting the release of acetylcholine. The neurotoxin is synthesized as a single-chain polypeptide of approximately 150 kDa; in this form the molecule has relatively little potency as a neuromuscular blocking agent. Toxicity is a result of a multistep mechanism: the single-chain molecule is proteolysed to form a di-chain molecule, in which the two chains (H-chain, 100 kDa; L-chain, 50 kDa, associated with one atom of zinc) remain linked by a disulphide bond[21,22]. This protein has three functional domains, where the catalytic function is confined to the L-chain (which acts as a zinc endopeptidase with proteolytic activity concentrated at the N-terminal end), the translocation activity to the N-terminal half of the H-chain, and receptor binding to its C-terminal half[21–24]. It is in this form that the toxin enters the axon terminal. The second activating step, disulphide reduction, occurs only after internalization by the target cell[22].

Botulinum neurotoxin binds to the presynaptic nerve endings of cholinergic neurones and enters by receptor-mediated endocytosis. The zinc-dependent catalytic domain of the seven serotypes specifically cleaves one of several different proteins essential for synaptic vesicle fusion. This cleavage results in inhibition of acetylcholine secretion, ultimately leading to paralysis[21–24]. Acetylcholine release is inhibited when the endopeptidase cleaves one or more neuronal proteins of the vesicle transport pathway[22–26]. The translocation of an intact vesicle from the cytosol to the plasma membrane requires a suite of specific proteins. Cleavage of any of these proteins impedes neurotransmitter release[27–30]. Intracellular substrate for both type B botulinum toxin and tetanus neurotoxin is the vesicle protein vesicle-associated membrane protein–synaptobrevin 2[31]. Types D and F botulinum toxin also cleave vesicle-associated membrane protein–synaptobrevin 2, but at a different site from B serotype[32]. Types A and E cleave another translocation protein, synaptosome-associated protein 25 kDa[28], and the C serotype acts by cleaving syntaxin.

After synaptic transmission has been blocked by botulinum toxin, the muscles become clinically weak and atrophic. The affected nerve terminals do not degenerate, but the blockage of acetylcholine release is irreversible[23]. Function can be recovered by the sprouting of nerve terminals and the formation of new synaptic contacts[20,23]; this usually takes 2–3 months.

Clinically, botulinum toxin doses must be tailored according to the mode of use and the individual patient[3,5]. Generally, the effective dose depends on the mass of muscle being injected[23], but susceptibility to the toxin varies. Some patients who respond well initially develop tolerance to the agent[33]. As indicated by Borodic *et al.*[34], sensitization is a major cause of decreased or absent clinical response after repeated injections and this can be caused by the development of neutralizing antibodies to the toxin.

Botulinum toxin injections are generally well tolerated. After injection, the toxin diffuses into the muscles and other tissues. Its effect diminishes with increasing distance from the injection site, but spread to nearby muscles is possible, particularly when large volumes are injected. Distant effects may also occur, but generalized weakness, possibly due to botulinum toxin spreading in the blood, is rare[3]. Nevertheless, this drug should be used with caution in patients with disturbed neuromuscular transmission, such as in myasthenia gravis or during treatment with aminoglycosides[23,33].

Chronic anal fissure

A chronic anal fissure is a 'cut' or 'crack' in the anal canal or anal verge that may extend from the mucocutaneous junction to the dentate line[8,9,35]. It shows great reluctance to heal without intervention. The majority of fissures occur in the posterior midline of the anal canal; multiple fissures or fissures occurring in the lateral position should raise suspicion of other diseases, including Crohn's disease, ulcerative colitis, human immunodeficiency virus infection and syphilis[8,9,35]. Classical symptoms are pain on or after defaecation that is often severe and may last from minutes to several hours. The patient may report that constipation is the antecedent event and, once pain has developed, fear of the act of defaecation may exacerbate the problem. However, a history of antecedent constipation is obtained in only one in four cases and diarrhoea is noted as a predisposing factor in about 6 per cent of patients[35]. Fissure is associated with childbirth in 3–11 per cent of patients, when it commonly occurs in the anterior midline[8,9,35] and is associated with reduced anal canal pressures[36].

The fissure can be seen as the buttocks are parted. It is often suspected because there is marked spasm of the anus, making examination difficult. Digital or proctoscopic examination is often impossible because of pain. Spasm of the IAS has been noted in association with chronic fissure, and for many years treatment has focused on alleviating IAS hypertonia.

Pathophysiology of chronic fissure

The cause of chronic fissure and the reasons for its failure to heal remain unclear. Also unexplained are the main characteristics of this painful condition, including the predilection for the posterior midline position and the lack of granulation tissue at the fissure site. Several theories have been advanced to unravel the underlying cause of anal fissure. Most are conflicting and none gives a satisfactory explanation for the characteristic features of the disease. Recognized features common to most chronic fissures are a high resting anal pressure due to IAS hypertonicity, reduced vascular perfusion index at the site of the fissure, and the presence of 'ultra-slow' pressure wave activity in the IAS[16,17]. It is generally believed that small traumatic tears in the lining of the anal canal fail to heal as a result of a reduced blood supply and so produce anal fissure.

It has been also found that the IAS of patients with anal fissure is fibrotic, compared with that of controls[37]; it was postulated that myositis might occur early in the course of the disease and that this was the underlying cause of both spasm and fibrosis. Furthermore, in patients healed by a conservative treatment, resting pressure tends to increase to pretreatment level while the patient remains symptom free. This probably means that sphincter hypertonicity does not necessarily cause pain.

It has been postulated that the increased incidence of fissure in the anterior and posterior midline positions is related to the distribution of vessels supplying blood to the anal canal. Relief of symptoms and healing induced by treatment might be attributed to a decrease in anal pressure, allowing an increase in mucosal blood flow and relief of ischaemia. The inferior rectal arteries branching from the internal pudendal artery provide the blood supply of the distal anal canal. These vessels cross the ischiorectal fossa; their divisions pass through the anal sphincters to reach the mucosa. Post-mortem angiography of the inferior rectal artery has revealed a paucity of inferior rectal artery branches at the posterior commissure in 35 of 41 subjects[38]. A morphological study of the capillaries revealed a reduced density in the subanodermal space and within the IAS in the posterior midline in most specimens. The predilection of anal fissures for the posterior midline and the lack of granulation tissue seen in the base of a chronic fissure may therefore be explained by ischaemia[39].

Decreased anodermal blood flow may be promoted by endothelial cell dysfunction associated with reduced nitric oxide synthesis, which is known to be involved in the regulation of local blood flow. Interruption of endothelial continuity not only removes the anticoagulant and vasodilatory functions of the endothelium, but also exposes the subendothelium, which promotes several procoagulant functions. In addition, even in the absence of detectable microscopic changes, endothelial function can change from vasodilatory to vasoconstricting, and from anticoagulant to procoagulant; these changes may be induced by inflammatory or immune cytokines. Activation of the endothelium may cause the expression of antigens; endothelial cells can act as antigen-presenting cells. Antiendothelial cell antibodies have been found in many patients with anal fissure but not in healthy controls[40]. In antibody-positive patients, higher resting anal tone has been observed[40]. The finding of circulating antiendothelial cell antibodies suggests that the lesion is primarily endothelial rather than at the level of the sphincter; a modification of RAIR was not observed in any of the positive patients[40]. This supports a role for the endothelium in the pathogenesis of anal ischaemia. Circulating antibodies may activate the endothelium to produce vasoactive autacoids, which could contribute to the increased basal tone and aggravate the ischaemia at the level of the posterior anal commissure. The observation that the topical application of glyceryl trinitrate (GTN) may induce healing of anal fissure in up to 60 per cent of cases supports a pathogenic role for endothelial nitric oxide synthesis[41,42].

A primary IAS disturbance may be a contributory aetiological factor. IAS supersensitivity to β_2 agonists has been observed in patients with chronic fissure[16,43]. This may be induced by a prolonged absence of the neurotransmitter, by abnormalities at neurotransmitter or metabolic level, or by a modification of cholinergic and adrenergic receptors. The efficacy of botulinum toxin in inducing fissure healing and reduction of resting tone suggests that increased IAS adrenergic or cholinergic activity is likely to occur in patients with chronic fissure[44–52].

Conservative therapies

As the passage of a hard stool is thought to contribute to the development of anal fissure, the control of constipation has long been considered a mainstay of treatment. Patients with a history suggestive of fissure of recent onset are often treated successfully by stool softeners, bulking agents, a high-fibre diet and Sitzbath[8,35]. To prevent recurrence, patients should be encouraged to continue with the conservative treatment, even after symptoms have resolved.

Anal dilators have also been employed in the treatment of anal fissure for many years. However, the addition of a dilator confers no benefit over stool softeners or topical anaesthesia alone[8,35]. It has been reported that more than half the patients can be initially cured using this technique, but half of those cured relapse[35].

Surgical treatment

If symptoms fail to resolve or have been present for a long period of time, resolution without surgery becomes increasingly unlikely. Lateral internal sphincterotomy has been the most commonly used surgical treatment for chronic fissure since the 1950s[53]. It may be performed under local or general anaesthesia, through a radial or circumferential incision, or using a subcutaneous approach. All methods produce an adequate fall in anal pressure. The IAS may be divided from medial to lateral, or vice versa. Results of this operation have been reported from many centres. Surgery is associated with several complications, most of which can be prevented by the use of a judicious technique and, of course, by familiarity with anorectal anatomy. Although sphincterotomy heals and relieves the symptoms of chronic fissure in nearly all patients (96 per cent)[54–57], the incidence of subsequent incontinence varies. The largest studies report impairment of continence in up to 30 per cent of patients and, although most episodes of incontinence are minor and transient, in a subset of patients incontinence is permanent[56].

Incision of the IAS throughout its whole length is inadvisable, but it is uncertain how much of the sphincter should be divided. A common practice is to divide it over the length of the fissure. It has been suggested that the length of the sphincterotomy does not affect the incidence of recurrence and the alterations of continence, but there is disagreement on this point[57–60].

Treatment with nitric oxide donors

Nitric oxide donors may promote the healing of anal fissure by increasing local blood flow in two ways: by reducing intra-anal pressure and by having a vasodilatory effect on the anal vessels. The indications for the use of nitric oxide donors have been documented[61–75]. Local application of nitro-derivates reduces anal pressures, improves anodermal blood flow[72] and significantly reduces pain within 5 min of application[41]. Between 19 and 58 per cent of patients experience transient headache when using topical GTN preparations and many have reported a burning sensation in the anus[41,61–75]. Benefit from GTN is not always permanent[66]. It has recently been reported that treatment with topical GTN is less efficacious than previously thought[65,73]. One study highlighted issues relating to poor patient compliance, and the authors considered that 62 per cent of their patients had side-effects that interfered with quality of life[65]. High doses of GTN are not more efficacious in inducing healing[67]. An important issue is the development of drug tolerance, which is well documented when the same drugs are used to treat cardiovascular diseases. Nitrates have a short duration of action and so frequent application is necessary[73]; side-effects, such as headaches and tachyphylaxis, further limit their usefulness[67]. Interestingly, there is no correlation between plasma GTN concentration, resting anal pressure, and the onset, duration or intensity of headache[75]. Recent studies suggest that GTN is labour intensive for patients and that its results are not superior to those of lateral internal sphincterotomy[70,71].

Alternative nitrates, such as isosorbide dinitrate, have been investigated. This agent (1 g 1 per cent ointment applied every 3 h throughout the day) achieved healing in 30 of 34 patients[76]. Similarly, healing was demonstrated in 34 of 41 patients following its topical use in a dose of 1·25 or 2·5 mg applied three times daily[77].

Calcium channel antagonists

Calcium channel antagonists, such as nifedipine, decrease anal sphincter tone if given sublingually or orally. In the first clinical study, 20 mg sublingual nifedipine caused (at 30 min) a 32 and 24 per cent reduction in anal canal pressure, respectively, in patients with anal fissure and normal volunteers, without any significant effect on blood pressure or heart rate[78]. An oral dose of 20 mg twice daily was used to treat 15 patients with chronic fissure[79]. The initial dose produced a 36 per cent fall in mean anal resting pressure, and healing was achieved by 8 weeks in nine of the 15 patients. The principal side-effect was flushing of the face and limbs, which was usually short-lived. The effectiveness of nifedipine retard 40 mg daily was assessed over a 5-day period in healing anal fissure[80]. Healing was complete in nine of 15 patients and symptomatic improvement was documented in a further three.

The effectiveness of topical diltiazem in decreasing anal pressure has been demonstrated recently[81–83]. In a pilot study, fissures healed in 10 of 15 patients using a 2 per cent gel without any reported side-effects[81]. Preliminary results suggest that this therapy does not have the significant side-effects associated with the oral preparation[82,83], and that topical diltiazem is more effective in achieving healing than topical nitrates and with fewer side-effects[83].

Muscarinic agonists and sympathetic neuromodulators

Muscarinic agonists promote nitric oxide synthesis in NANC neurones. Bethanechol, used as a 0·1 per cent topical cream, decreased sphincter pressure and healed fissures without side-effects[81]. Rates of healing may be similar to those with GTN and diltiazem.

Sympathetic neuromodulators, such as oral indoramin (an α_1 antagonist) and salbutamol (a β_2 agonist), have been shown to reduce anal pressure by relaxing the IAS in healthy volunteers and in patients with fissure[18]. The effectiveness of these drugs in the treatment of fissure has yet to be evaluated.

Botulinum toxin treatment

Botulinum toxin can be used to treat anal fissure (*Table 1*), particularly when there is a high risk of incontinence. With the patient lying on one side (usually the left side when the operator is right-handed), the lower rounded edge of the

Table 1 Published results of the treatment of chronic anal fissure with botulinum neurotoxin

Reference	Year	No. of patients	Name of drug†	Dose (units)	Healing rate at 6–8 weeks (%)	Temporary incontinence (%)	Recurrence (%)	Complications (%)
Gui *et al.*[44]	1994	10	Botox	15	70	10	20	10
Jost and Schimrigk[45]	1994	12	Botox	5	83	0	8	0
Jost *et al.*[84]	1995	54	Botox	5	78	6	6	11
Mason *et al.*[46]	1996	5	Dysport	—	60	0	0	0
Jost[47]	1997	100	Botox	5	82	7	6	0
Espi *et al.*[85]	1997	36	Botox	10–15	65–81	0	0	0
Maria *et al.*[48]	1998	25	Botox	20	88	4	0	0
Maria *et al.*[49]	1998	57	Botox	15–20	44–68	0	0	0
Minguez *et al.*[50]	1999	69	Botox	10–21	48–70	0	37–52	0
Jost and Schrank[86]	1999	50	Dysport	20–40	76–80	4–12	4–8	0
Brisinda *et al.*[42]	1999	25	Botox	20	96	0	0	0
Fernandez Lopez *et al.*[87]	1999	76	Botox	40	67	3	0	1
Maria *et al.*[51]	2000	50	Botox	20	74	0	0	0
Lysy *et al.*[88]*	2001	30	Botox	20	73	0	0	—
Madalinski *et al.*[89]*	2001	14	Botox	25–50	54	0	8	—
Brisinda *et al.*[52]	2002	150	Botox	20–30	89–96	3	4	0

*Plus topical nitrates. †Dysport® is trade name of the type A botulinum toxin preparation manufactured by Ipsen (Maidenhead, UK); Botox® is the trade name of the type A preparation manufactured by Allergan (High Wycombe, UK). There has been much confusion over the doses and units of potency of the two preparations; although doses are quoted in mouse units (the amount of toxin that kills 50 per cent of a group of 18–20-g female Swiss–Webster mice), implying some standardization, Botox® seems to be more potent. It is suspected that 1 unit Botox® is equivalent to 3–5 units Dysport®; a Dysport®: Botox® equivalency ratio of approximately 3 : 1 or 4 : 1 at standard vial dilutions has been suggested

IAS can be felt on digital examination about 1–1·5 cm from the dentate line, and is easily injected.

In an open-label study, Gui *et al.*[44] observed that six of ten patients healed following a single infiltration of 15 units into the IAS. In a subsequent double-blind study, a success rate of 76 per cent was achieved following a single treatment with 20 units[48]. A prospective comparison between two dose regimens (15 and 20 units) showed negligible side-effects and no complications; symptomatic improvement was achieved in both groups of patients, but the healing rate was higher in the group receiving 20 units[49]. The present authors have also been able to demonstrate that fissure healing is induced more efficiently by botulinum toxin treatment than by GTN, and that IAS hypertonia is also alleviated more effectively[42]. Compared with baseline values, 2 months after treatment, resting anal pressure was reduced by 28 per cent in the group of patients receiving toxin and by 14 per cent in those receiving GTN. Recently, it has been noted that topical isosorbide dinitrate potentiates the effect of botulinum toxin in patients with refractory chronic fissure[88].

The therapeutic efficacy of different botulinum toxin doses in chronic fissure has been reported recently[50,52]; the rate of healing did not differ significantly when the total dose and the number of injection sites were varied. The healing rate was 83 per cent with 10 units, 78 per cent with 15 units and 90 per cent with 21 units[50]. Between-group comparison did not reveal significant differences. The injections were administered through the intersphincteric groove in the direction of the IAS; however, 1 month after treatment the mean squeeze pressure was reduced more than resting pressure, suggesting diffusion of the toxin to the EAS[50]. Based on the theory that low anodermal perfusion at the base of the fissure contributes to the pathophysiology, additional infiltration of botulinum toxin has been performed at this point[50].

In the authors' experience, patients with a posterior fissure have better results, represented by a lowering of resting anal tone and early development of a healing scar, when botulinum toxin is injected anteriorly into the IAS[42,51,52]. Anteriorly placed injections induce a higher fall in resting pressure and improve clinical outcome. Fibrosis of the IAS, which is more prominent at the site of the fissure than elsewhere in the smooth muscle, may reduce IAS compliance and limit the diffusion of toxin. It is known that the myenteric plexus with myenteric ganglia is located between the circular and longitudinal smooth muscle layers along the entire extent of the IAS. A chronic reduction of perfusion in the posterior part of the anus may affect the myenteric nervous fibres at this location and make them less sensitive to the action of botulinum toxin.

In a recent study, the influence of two different dosage regimens injected anteriorly in the IAS on the clinical outcome of patients with a posterior chronic fissure was investigated[52]. Fissure healing and symptomatic improvement were achieved in both groups of patients. Two months after injection, 75 patients (89 per cent) treated with 20 units and 75 patients (96 per cent) treated with 30 units had a healing scar; three patients in the latter group had a persistent fissure in the absence of symptoms. These results suggest that higher doses led to a higher success rate[52]. Resting anal pressures were significantly lower than pretreatment values in both groups; although maximum voluntary pressure was unchanged in patients treated with 20 units, it was significantly lower than the pretreatment value in patients treated with 30 units, probably related to a diffusion of toxin to the EAS. Five of the 75 patients receiving 30 units reported mild incontinence of flatus that lasted for 2 weeks after treatment and disappeared spontaneously. Diffusion of toxin in the tissues is a dose-dependent phenomenon; histochemical staining of acetylcholinesterase suggested that higher doses of botulinum toxin produced a biological effect throughout the entire muscle, whereas smaller doses produced a gradient down the length of the muscle studied.

It has been observed that botulinum toxin injections into the EAS are also effective for treating fissure[47,84,86]. The mechanism is probably mediated by diffusion to the IAS, as shown by the observation that maximum squeeze pressure and resting pressure are both reduced following this procedure. Since the fundamental pathogenic event in chronic fissure is IAS spasm, injection into the EAS cannot be the first choice of treatment. In addition, the IAS is readily identifiable and easier to inject than the EAS. In a series of 50 patients who received 10 or 20 units into the EAS adjacent to the fissure margins, 78 per cent had healed by 3 months[86]. There was no significant difference in healing rates between the two groups, but patients receiving the higher dose showed a greater reduction in sphincter tone in the puborectalis muscle, and a higher incidence of transient incontinence and other complications[84].

Chronic constipation due to pelvic floor dysfunction

Constipation is one of the commonest chronic complaints. Idiopathic constipation can be classified into two pathophysiological types: slow-transit constipation and pelvic floor dysfunction. Pelvic floor dysfunction is characterized by a failure of the puborectalis muscle to relax during efforts to defaecate, or by its paradoxical contraction. With an effort to evacuate the rectum, the puborectalis and the EAS normally relax to straighten the anorectal angle and open the anal canal. The diagnosis of pelvic floor dysfunction is suggested by the demonstration of a persistent impression of the puborectalis on the posterior surface of the anal canal during attempted evacuation of barium paste and, more reliably, by electromyographic evidence of increased electrical activity in the puborectalis muscle during straining.

The aetiology of pelvic floor dysfunction is unclear. Prolonged efforts to empty the rectum may aggravate the condition. It has been suggested that paradoxical puborectalis contraction during straining represents a focal dystonia, characterized by excessive recruitment of synergistic and antagonistic muscle groups during voluntary activity and lack of reciprocal inhibition[90]. Signs of anismus have been noted in normal subjects, in patients with anorectal pain, and in patients with faecal incontinence[91–93].

Botulinum toxin has been used selectively to weaken the EAS and puborectalis muscle in constipated patients. It has been observed that the toxin relaxes the puborectalis muscle[94] and, as a consequence, the anorectal angle increases during straining and evacuation becomes possible. However, despite good results, the effect of botulinum toxin injections is fairly short-term. In one study, 6–15 units were injected under electromyographic guidance into the EAS or the puborectalis muscle[95]; while this brought about short-term improvement of all four treated patients who had failed to respond to conventional biofeedback therapy, long-term success occurred in only two. In a more recent study, symptomatic improvement was noted in 13 of 15 patients following the injection of 25 units into the EAS[96]. Improvement was maintained for a mean of only 5 months and so reinjection was necessary.

Of a group of 50 patients with outlet obstruction constipation, four with a puborectalis syndrome were studied using anorectal manometry, defaecography and electromyography. They were then treated with a total of 30 units, injected at two sites on either side of the puborectalis muscle, under ultrasonographic guidance[97]. One patient was lost to follow-up; in the remaining three, the frequency of natural bowel movements increased from 0 to 6 per week and only one patient needed laxatives. Anorectal manometry demonstrated decreased tone during straining from a mean(s.d.) of 96(12) to 42(13) mmHg at 4 weeks ($P = 0.003$) and 63(22) mmHg at 8 weeks ($P = 0.009$). Defaecography performed 8 weeks after treatment showed improvement in the anorectal angle, which increased from a mean(s.d.) of 94(11) to 114(13)°; $P = 0.01$), and evacuation of barium paste.

Outlet-type constipation may also occur in Parkinson's disease. Following the observation of a dramatic improvement of outlet-type constipation in a patient treated with botulinum toxin injections into the puborectalis muscle[98], a prospective study was undertaken to identify the prevalence of this condition among outpatients with Parkinson's disease. Of 120 patients who met the inclusion criteria for chronic constipation, 13 (11 per cent) had isolated or combined outlet-type constipation. Ten of these patients (one woman), of mean(s.d.) age 70(8) years, were enrolled in a prospective study[99]. They received botulinum toxin injections into the puborectalis, the total dose per session being 30 units (three patients), 60 units (one patient) or 100 units (six patients). The results indicated a significant reduction of tone during straining, from a mean(s.d.) of 95(42) mmHg at baseline to 38(11) mmHg at 1 month, and 38(9) mmHg at 2 months. There was also an improvement in the anorectal angle, which was increased from 98(8)° before treatment to 124(15)° ($P < 0{\cdot}05$)[99].

Anterior rectocele

Rectocele is a hernia of the anterior rectal wall into the lumen of the vagina. It is a frequent finding in women and its clinical relevance is questionable; between 20 and 81 per cent of asymptomatic and constipated women may present with a rectocele[100,101]. If less than 2 cm in diameter, rectocele is considered a normal finding in constipated patients. When the diameter increases beyond 2 cm, the rectocele may cause outlet obstruction and rectal emptying difficulties[100–102].

It has been suggested that in some instances the rectocele is caused by failure of relaxation or paradoxical contraction of the puborectalis muscle during attempted evacuation, but the reason for its establishment is not clear. It is conceivable that straining aggravates the rectocele, enlarges it, and makes evacuation even more difficult. When there is a rectocele or a paradoxical sphincter reaction, defaecation must occur through the non-relaxed pelvic floor[100–103].

Although simple to diagnose, rectocele has proven difficult to treat. A wide variety of surgical approaches has been proposed with the aim of ensuring rectal emptying by reducing the dimension of the rectocele. However, the results of surgery are often disappointing with regard to evacuation. Surgical repair, either vaginal, transperineal or transanal, does not always alleviate symptoms, and in some patients causes impaired faecal continence. Furthermore, transanal repair may compromise anal sphincter pressures and an alternative approach should be considered when the anal sphincter is lax. Some 30–72 per cent of patients still have difficulties with evacuation in the postoperative period. In one study 54 per cent of patients still complained of constipation, often severe, after this surgical procedure; 17 per cent had persistent anorectal pain and 34 per cent had impaired faecal continence. Six months after successful transanal repair, a significant impairment of resting and squeezing anal pressures, probably due to stretch of the anal sphincters, was still detectable[100]. An increased incidence of rectovaginal fistula and vaginal narrowing has been reported when the transvaginal approach is used.

Recently, 14 women were treated with a total of 30 units botulinum neurotoxin evenly divided into three sites, two on either side of the puborectalis muscle and the third anteriorly in the EAS[104]. At 2-month evaluation, a symptomatic improvement was found in nine patients. At defaecography, the rectocele depth was reduced from a mean(s.d.) of 4·3(0·6) to 1·8(0·5) cm ($P < 0{\cdot}001$) and the rectocele area was reduced from 9·2(1·3) to 2·8(1·6) cm^2 ($P < 0{\cdot}001$). The anorectal angle measured during straining increased from a mean(s.d.) of 98(15)° before treatment to 121(19)° ($P = 0{\cdot}001$).

Chronic idiopathic anal pain

Chronic idiopathic anal pain is part of a rather ill-defined group of disorders termed chronic idiopathic perineal pain, which also includes proctalgia fugax and coccygodynia[105–107]. The main feature of these syndromes is that no objective abnormality can be found on clinical examination, and the distinction between the different groups of perineal pain is based solely on the patient's description of the pain and the location of tenderness by palpation. Patients usually describe a pain located at the anus, often with radiation to the upper anal canal or lowest part of the rectum. In the majority, pain is present constantly and is usually intense and sometimes burning, often with some irradiation. It is usually aggravated by sitting and relieved by lying down, while defaecation has no constant effect[105–107]. The cause and pathogenesis of the syndrome are unknown, but in a few patients more advanced, instrumental investigation of the anal canal, such as intra-anal ultrasonography and magnetic resonance imaging, may reveal a treatable organic disorder, such as chronic intrasphincteric abscess or fistula.

There is no satisfactory treatment for chronic anal pain, although anal stretch and lateral internal sphincterotomy are still used sometimes on the assumption that the pain might be caused by a hypertonic IAS. Recently, injections of botulinum toxin (20 units placed intersphincterically in four quadrants and/or at the lower rim of the puborectalis muscle under ultrasonographic guidance) have been described to result in improvement in four patients with chronic anal pain; this improvement persisted during a follow-up interval of 12–24 months[107].

Conclusion

Botulinum toxin appears to be a safe treatment for anal fissure. It is less expensive and easier to perform than surgery and does not require anaesthesia. It is also more efficacious than nitrate therapy and its benefit is not related to a patient's willingness to complete ongoing treatment. For posterior chronic fissure, better results are achieved when botulinum toxin is injected anteriorly into the IAS. No adverse effects or permanent IAS damage have resulted from botulinum toxin injection. The authors believe that this treatment should be considered as first-line therapy for chronic anal fissure.

Botulinum toxin also appears to be a promising approach for the treatment of chronic severe constipation due to pelvic floor dysfunction. No adverse systemic effect or permanent sphincteric damage has been reported to result from injection of the toxin, which seems to relieve the contraction of the puborectalis muscle and allow resumption of normal rectal voiding. The use of higher dosage and the availability of a more precise method of toxin injection by means of transrectal ultrasonography account for an improved long-term success rate. Nevertheless, further investigation of the efficacy and safety of botulinum toxin treatment in this context is indicated before its general usage can be advocated.

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Review

Liver fluke-associated cholangiocarcinoma

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Background: Infection with liver flukes has been reported to be associated with bile duct malignancy.

Methods: The review is based on a literature search (Medline) and, in some cases, direct contact with authors or principal investigators.

Results: A large body of evidence indicates that *Opisthorchis viverrini* is a definite cause of human cholangiocarcinoma, whereas *Clonorchis sinensis* is a probable cause. The evidence regarding *Opisthorchis felineus* is insufficient to assess its role in carcinogenesis. Possible mechanisms of carcinogenesis include chronic irritation, nitric oxide formation, intrinsic nitrosation and activation of drug-metabolizing enzymes. Early detection of bile duct malignancy is difficult and not clinically available at present, although cholangiocarcinoma-associated soluble antigen has been reported in an experimental study to be a useful early marker of cancer development. Long-term survival after surgical treatment of liver fluke-associated cancer is similar to that reported in patients without liver fluke infestation.

Conclusion: Liver fluke-associated cholangiocarcinoma is still a health problem in developing countries. Mechanisms of carcinogenesis should be explored further in order to reduce the impact of this disease.

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Extent of the problem

According to the 1994 report of the World Health Organization and the International Agency for Research on Cancer (IARC) meeting[1,2], crude estimation of the global number of liver fluke infestations is of the order of 17 million, comprising 9 million with *Opisthorchis viverrini*, 7 million with *Clonorchis sinensis* and 1·5 million with *Opisthorchis felineus*. *O. viverrini* infestation is found predominantly in Thailand, Laos and Cambodia, whereas *C. sinensis* is endemic in southern China, Korea, Taiwan and Vietnam. *O. felineus* is endemic in western Siberia, Kazakhstan, Ukraine and parts of eastern Europe. Recent recovery of *O. felineus* from cyprinoid fish and cats in Germany indicates the persistent existence of the fluke in Europe[3].

Association between liver fluke and cholangiocarcinoma: clinical studies

Evidence of association between *O. viverrini* and bile duct malignancy has emerged from several studies, including hospital-based case series, population-based ecological studies that correlated the incidence of cancer with prevalence of infestation in various geographical areas, and a few case–control studies. The first large case series was reported from Siriraj Hospital, Mahidol University, in Bangkok[4]. The authors analysed 9694 autopsies performed between 1954 and 1965, and 1301 liver biopsies obtained between 1960 and 1962. An unusually high incidence of cholangiocarcinoma was observed in both the autopsy and biopsy materials taken from patients with *O. viverrini* infestation. The ratio between hepatocellular carcinoma and cholangiocarcinoma in autopsies without opisthorchiasis was 8 : 1, whereas the ratio was reversed among those with liver fluke infestation. Similarly, the ratio of these two malignancies in biopsies was 5 : 1 in non-infected patients and 1 : 2 in the presence of the fluke.

A nationwide survey of liver pathology in Thailand by Bunyaratvej and colleagues, who examined 3305 biopsy specimens collected between 1974 and 1978, also demonstrated a high incidence of cholangiocarcinoma in association with opisthorchiasis in the north-east of Thailand[5]. More than two-thirds of the patients were male, and the mean age at diagnosis of cholangiocarcinoma was 48·8 years in males and 48·1 years in females. The results of this study were later confirmed by Srivatanakul *et al.*[6], who showed that the incidence of cholangiocarcinoma was almost twice that of hepatocellular carcinoma in endemic areas of *O. viverrini* in the north-east of the country, and the incidence in males was 2·4 times that in females. Subsequent study found that the highest proportional incidence rate of cholangiocarcinoma was in the north-eastern region, where the prevalence of *O. viverrini* infestation was also highest[7].

In a population-based study in a fluke-endemic area, the age-standardized incidence rates for the year 1988 were 89·2 per 100 000 in males and 35·5 per 100 000 in females – the highest rates of cholangiocarcinoma in the world. Moreover, the tumour burden appeared to correspond with the intensity of *O. viverrini* infestation[8]. Studying in the same year and in the same region by using hospital-based data, Green *et al.*[9] estimated the age-standardized incidence rates of cholangiocarcinoma to be 135·4 and 40·0 per 100 000 in males and females respectively. The truncated standardized incidence rates (in those aged 35–64 years only) were 334·2 per 100 000 in males and 104·3 per 100 000 in females. Parkin and colleagues[10] performed a case–control study of 103 patients in comparison with controls matched by age and sex. They found that *O. viverrini* infection increased the risk of cholangiocarcinoma fivefold, and that males carried a higher risk than females. They estimated that two-thirds of cases of cholangiocarcinoma in Thailand were caused by *O. viverrini* infestation.

In a longitudinal study of clinical, laboratory and hepatobiliary ultrasonography of 913 voluntary subjects infected with *O. viverrini*, treatment with the antihelminthic drug praziquantel significantly improved clinical symptoms and ultrasonographic changes[11]. A study in Syrian hamsters, however, showed that removal of the parasite load in itself might not be sufficient to block the development of cholangiocarcinoma, particularly if the fluke had already caused significant and permanent changes in the biliary system[12]. There are no concrete data available to confirm the benefit of fluke eradication in terms of reducing cancer death.

An association between *C. sinensis* and cholangiocarcinoma was first made by Katsurada in Japan, just over a century ago[13]. Although interest in the relationship between the fluke and the cancer was initially stimulated by a series of reports by Hou in Hong Kong[14–16], the disease is uncommon in Hong Kong today. By contrast, clonorchiasis-associated cholangiocarcinoma is still a problem in Korea. In Pusan, an area with extremely high prevalence of *C. sinensis*, the fluke increased the risk of cholangiocarcinoma sixfold[17]. More recently, a case–control study[18] in the same area showed that identification of *C. sinensis* in the stool was significantly associated with cholangiocarcinoma, with a relative risk of 2·7. Cases of cholangiocarcinoma associated with *C. sinensis* have also been reported among Asian immigrants to the USA[19,20].

There are several case reports or case series of cholangiocarcinoma in patients with *O. felineus* infestation[21–23], but fewer than a handful of systematic studies have been conducted to identify the actual relationship between the two conditions[1].

Association between liver fluke and cholangiocarcinoma: experimental studies

An experimental model successfully demonstrating an association between liver fluke and cholangiocarcinoma was obtained using a combination of liver fluke infestation and continuous administration of a hepatocarcinogen in the drinking water or the basal diet of hamsters. Examples are dimethylnitrosamine (DMN) in the drinking water for 10 weeks plus *O. viverrini*[24,25]; DMN in drinking water for 8 weeks plus *C. sinensis*[26]; 0·03 per cent *N*-2-fluorenylacetamide in the basal diet for 40 weeks plus *C. sinensis*[27]. In hamsters receiving DMN (0·0025 per cent in drinking water) and 100 metacercariae of *O. viverrini*, more than 50 per cent of the hepatic parenchyma was occupied by cholangiocellular lesions and all animals developed cholangiocarcinoma[24]. Similar increases in cholangiocarcinoma, as well as preneoplastic hepatocellular lesions, were also found in hamsters receiving continuous administration of combined nitrite and aminopyrine in drinking water and *O. viverrini*[28]. The report of DMN formation in the stomach by nitrite and aminopyrine may explain such findings[29].

Based on a critical review of existing evidence from several clinical and experimental studies, the working group of IARC concluded that *O. viverrini* is a definite cause of bile duct cancer in humans; *O. viverrini* infestation is therefore classified as a carcinogen. The review reported that *C. sinensis* is a probable cause; *O. felineus* has not yet been studied sufficiently to assess its carcinogenic role[1].

Pathological studies

Infestation of liver flukes occurs when humans or other mammals ingest raw fish containing the infective cysts of the fluke (metacercariae) in the muscle and connective tissue. After being eaten by humans, the infective metacercariae excyst in the duodenum. The newly excysted flukes move into the bile duct through the ampulla of Vater and develop into adults in the intrahepatic bile ducts, and occasionally in the pancreatic duct or gallbladder, within 1 month. The life cycles of *O. viverrini*, *C. sinensis* and *O. felineus* are similar. The adult fluke lays eggs that enter the biliary system and are excreted in the faeces, to be ingested by snails of *Bithynia* species, the first intermediate hosts. The eggs hatch inside the snail and mature, eventually leaving the snail in the form of freely swimming cercariae that find and penetrate cyprinoid fish, the second intermediate hosts, where they develop into metacercariae in the muscle and connective tissue. The lifespan of *O. viverini* and *C. sinensis* has been reported to be 25–30 years[30–32].

Pathological consequences of *O. viverrini* infection appear to be similar in both animals and humans[33,34]. Early pathological changes consist of an acute inflammatory reaction involving the bile ducts of the second order and the portal connective tissue, particularly the large veins. Death of bile duct epithelial cells and hepatocytes, and evidence of cell regeneration, occur early after liver fluke infestation[33,35]. As the flukes develop into adults, they induce hyperplasia of the bile duct epithelium and adenoma formation. The adult flukes and ova also induce a granulomatous reaction. Resolution of such granulomas leads to periductal and portal scarring. Bile ductule proliferation associated with periportal fibrosis and linking up between portal areas by fibrous bands results in the appearance of multilobular cirrhosis in most hepatic lobules. The periportal and periductal fibrosis in hamsters with chronic infection of *O. viverrini* has been shown to correlate with a marked increase in the synthesis and hepatic content of type I and type III collagen[36]. In the liver of patients with opisthorchiasis, the subcapsular bile ducts are usually dilated, with prominent fibrotic wall[34,37]. Microscopically, pathological changes in the liver are confined to the biliary tree, particularly the large and medium-sized bile ducts where the flukes are usually found[34]. Moreover, obstruction of bile ducts due to large numbers of the flukes in the ductal lumen is frequently seen. This obstruction may lead to suppurative cholangitis with abscess formation, and cholangiohepatitis may subsequently develop, particularly when rupture of abscess occurs[35].

In hamsters receiving both the carcinogen DMN (0·0025 per cent in drinking water) and 100 metacercariae of *O. viverrini*, all animals developed mucin-producing cholangiocarcinomas, with or without cholangiofibrosis[24]. When hamsters received a subcarcinogenic oral dose of *N*-nitrosodimethylamine (1·6 mg) and 50 metacercariae of *O. viverrini*, 20 per cent of the animals developed cholangiocarcinoma. All tumours were of intrahepatic type and were found most frequently in the right lobe[38]. In humans, however, the tumour location has been reported to be of central type (60 per cent), peripheral type (20 per cent) and diffused type (20 per cent)[40]. Of the extrahepatic lesions, 60 per cent are located in the proximal ductal region and are of the scirrhous type, with an occasional papillary component. Nodular and papillary types are found in the middle and distal ductal region[40,41]. The histopathology is almost always adenocarcinoma and occasionally the flukes are found in the tumour or in the adjacent liver parenchyma (*Fig. 1*). Direct invasion and local metastasis via lymphatics, blood vessels and perineural are common, especially in extrahepatic cancer[42,43]. Among 29 non-jaundiced patients with opisthorchiasis and intrahepatic or peripheral-type cholangiocarcinoma, 23 had tumours in the right lobe[41].

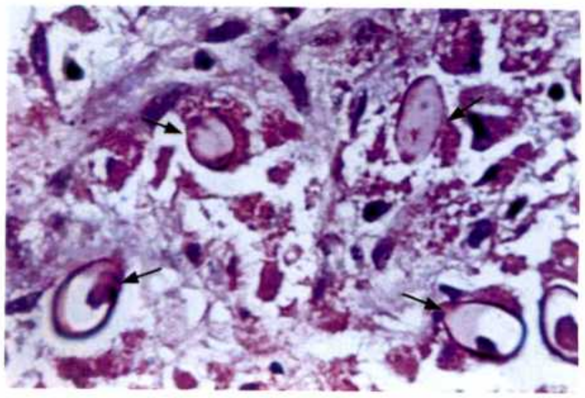

Fig. 1 Histological appearance of peripheral type of *Opisthorchis viverrini*-associated cholangiocarcinoma. Arrows show the eggs of the fluke in the area adjacent to the tumour. (Courtesy of Dr K. Atisook, Department of Pathology, Faculty of Medicine, Siriraj Hospital)

Scanning electron microscopy of a cholangiocarcinoma cell line (HuCCA-1), originally established from an intrahepatic bile duct tumour of a patient seropositive for antibody to *O. viverrini*, showed the surface of the cells to be covered with microvilli of varying size and irregular distribution. Transmission electron microscopy clearly revealed the presence of cytokeratin filaments, an intracytoplasmic lumen, tight junctions at the apices and desmosomes at the lateral surfaces of neighbouring cells, all of which are characteristics of an adenocarcinoma cell. Cells from a tumour mass that developed in a nude mouse following subcutaneous injection of HuCCA-1 cells exhibited some morphological changes. Nearly one-third of the tumour cells, particularly those lining the base of the tumour tubules, formed small foci of cells in sheet-like arrangements and exhibited electron-dense tonofilament bundles typical of squamous cells[44]. These findings are consistent with evidence from a clinicopathological study of an intrahepatic cholangiocarcinoma with a squamous cell carcinoma component[45].

Seventy per cent of *Clonorchis*-associated cholangiocarcinomas are adenocarcinomas and may be found at varying stages of differentiation[14]. Anaplastic tumours of the bile duct epithelium have been reported occasionally, but they are uncommon. They may resemble primary liver cell carcinoma[13]. Newly formed goblet cells are frequently found and mucin production may be abundant, but glycogen and bile pigment are characteristically absent.

Squamous metaplasia is found in 13 per cent of cases[14]. The tumour usually develops in the second-order intrahepatic bile ducts near the hepatic hilum. Adenomatous proliferation of ductal epithelium can often be seen side by side with malignant changes, and flukes are frequently found in bile ducts that are surrounded by tumour[46]. Intrahepatic or peripheral cholangiocarcinoma is common and is found much more often (as in *O. viverrini*-associated cancer) in the right lobe of the liver[47].

Molecular studies of carcinogenesis

Molecular mechanisms of human cholangiocarcinogenesis secondary to liver fluke infestation are still to be investigated, particularly in patients with *O. viverrini* infection. By using the polymerase chain reaction and direct sequencing of the product of c-Ki-*ras* gene, one study showed that five of nine Japanese patients with cholangiocarcinoma in normal liver (without associated opisthorchiasis) had a point mutation at codon 12, whereas none of 12 Thai patients with opisthorchiasis had such a mutation[48]. A more recent study confirmed the findings: the incidence of *ras* mutation differed markedly between the Japanese (seven of 12 patients without opisthorchiasis) and Thai (two of 26 with opisthorchiasis) patients. In contrast, the incidence of *p53* mutation was similar: four of 12 and nine of 26 respectively. All but one of the *ras* mutations occurred at codon 12 or 13 of the c-Ki-*ras* gene. The exceptional case was found to have deletion–insertion in the second exon of the N-*ras* gene. For *p53* mutations, all but one were detected in a highly conserved region, and the predominant form of the mutations was G : C → A : T transition at CpG sites in both the Japanese and Thai patients[49].

Results of such molecular studies on carcinogenesis in patients with liver flukes may pave a way for further investigations to crystallize the actual or causal relationship between the parasite and the tumour. However, the fact that the incidence of cholangiocarcinoma is low, in sharp contrast to the high prevalence of liver fluke infestation, even in fluke-endemic areas, highlights the importance of co-factors. Exploration of the molecular carcinogenesis of the tumour with several plausible aetiological factors will obviously be a lengthy and laborious task.

Possible mechanisms of carcinogenesis

The pathogenesis of liver fluke-associated cholangiocarcinoma may be a complex process, involving several mechanisms. From existing evidence, possible mechanisms may be suggested.

Hyperplasia of bile duct epithelium and carcinogen exposure

Chronic irritation and chronic inflammation caused by the fluke results in hyperplasia and adenomatous changes of bile duct epithelium[33,34]. As previously shown in malignancy of the colon or pancreas, hyperplastic cells are vulnerable to carcinogen because the agent can easily induce DNA damage during active cell proliferation[50,51]. If the DNA damage involves cell cycle control genes, neoplastic changes occur. It has been demonstrated that nitrates, nitrites and even *N*-nitroso compounds are commonly found in several fermented or preserved foods, and that these compounds can act as exogenous carcinogens[52,53]. Moreover, patients with liver fluke infestation have increased rates of endogenous nitrosation[54,55].

Increased formation of endogenous carcinogen

Endogenous nitrosation caused by liver fluke infestation has been studied in both animals and humans. In hamsters with *O. viverrini* infestation, induction of nitric oxide synthase in macrophages, mast cells and eosinophils in inflamed areas surrounding the bile ducts has been reported, and the enzyme subsequently increases endogenous nitrosation of thiazolidine-4-carboxylic acid (thioproline)[56]. In a dietary control (low-nitrate diet) study comparing patients with opisthorchiasis and uninfected controls, those with liver fluke infestation had increased endogenous generation of nitric oxide and production of *N*-nitroso compounds, as indicated by increased levels of plasma and urinary nitrate, salivary nitrite and increased nitrosation of proline and thioproline. Such increases were abolished by co-administration of ascorbic acid with proline, and by elimination of the fluke by treatment with the antihelminthic drug praziquantel. Production of *N*-nitroso compounds occurs through a reaction between amines and nitrosating agents derived from the oxidation of nitric oxide outside the stomach[55,57]. It is likely that *N*-nitroso compounds are formed in the area of chronic inflammation around the bile ducts as the result of local generation of nitric oxide by inflammatory cells. Therefore, the bile duct epithelial cells are exposed continuously to high concentrations of *N*-nitroso compounds, leading to neoplastic transformation. To prove this hypothesis, the concentration of *N*-nitroso compounds in the bile ducts should be assayed. However, measuring these compounds in bile is technically difficult at present, because they are labile and other components in bile can affect the assay.

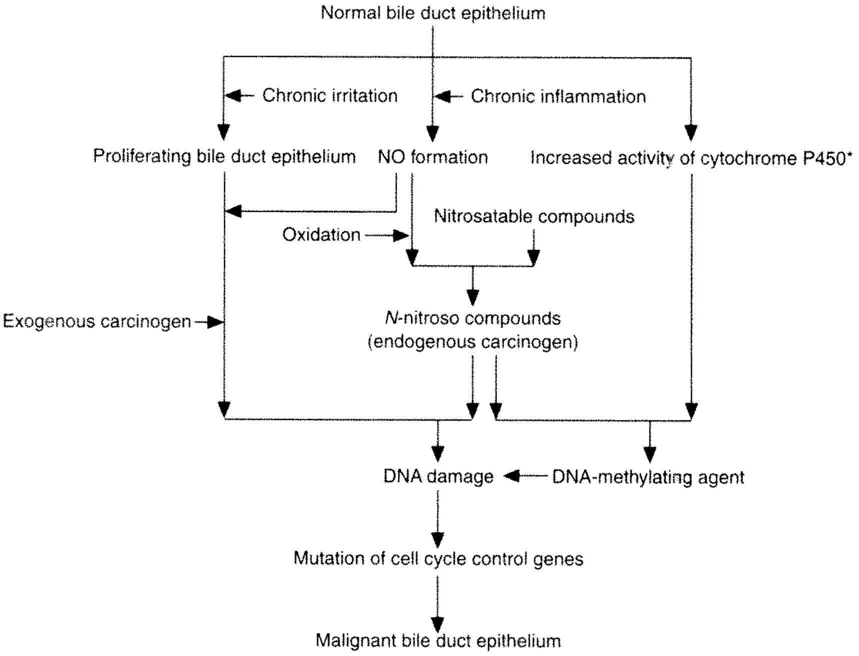

Fig. 2 Possible mechanisms of carcinogenesis of liver fluke-associated cholangiocarcinoma. *Particularly CYP2A6 and CYP2E1. NO, nitric oxide

Activation of drug-metabolizing enzymes

In male hamsters infected by *O. viverrini*, activities of the hepatic cytochrome P450 (CYP) isoenzymes (particularly CYP2E1 and CYP2A6) have been shown to be higher than those of controls, and the highest levels of these two enzymes occur in hepatocytes immediately adjacent to the area of inflammation[58]. Induction of CYP2A6 expression has been demonstrated in patients with opisthorchiasis, and a significant reduction of the enzyme activity was observed 2 months after eradication of the flukes by praziquantel treatment[59]. *N*-nitrosodimethylamine (NDMA), one of the products of endogenous nitrosation formed in the tissue, is significantly metabolized by CYP2E1 and CYP2A6. The product of this metabolism is a DNA-methylating agent that can result in DNA damage, particularly in proliferating bile duct epithelial cells. However, the mechanism of increased activity of these two enzymes in patients with chronic liver fluke infestation is as yet uncertain.

Increased nitric oxide production

In areas of chronic inflammation caused by the liver fluke, macrophages and other cell types (e.g. mast cells, eosinophils), activated by parasite-specific T cells and cytokines, synthesize nitric oxide from L-arginine via the induction of the enzyme nitric oxide synthase[56,60,61]. Nitric oxide is not only cytotoxic, but also genotoxic at physiological concentrations[62,63]. Chronic exposure of the proliferating bile duct epithelium to this genotoxic inflammatory product may create an environment favouring malignant change.

It is probable that the above four mechanisms of carcinogenesis may act in concert during the development of liver fluke-associated cholangiocarcinoma. The possible process of carcinogenesis is outlined in *Fig. 2*.

Early detection of cancer

A soluble antigen for early detection of *O. viverrini*-associated cholangiocarcinoma has been reported in hamsters[64]. This antigen is a 200-kDa glycoprotein that appears to be immunologically distinct from other tumour markers. The level of the antigen in serum and bile was raised markedly in animals with progressive tumours, compared with controls. Serum taken serially from each animal that subsequently developed cholangiocarcinoma showed a gradual but significant increase in the level of antigen as carcinogenesis progressed. Identification of the antigen, and the establishment of a cell line from *O. viverrini*-associated cholangiocarcinoma induced in a hamster model and a cholangiocarcinoma cell line from a patient with liver-fluke infestation, seem to provide an early opportunity for tumour detection[65,66]. However, identifying a tumour marker in serum or bile does not, on its own, solve the problem. The intrahepatic biliary system is a complex tubular structure. Although abdominal computed tomography (CT) (particularly three-dimensional spiral CT cholangiography) or magnetic resonance imaging can detect cholangiocarcinoma with an accuracy of up to 90 per cent[67,68], the demonstration of a small focus of early cancer change in the liver is difficult. Confirmation of neoplastic change from such small lesions is difficult, particularly when the tumour is beyond the second-order bile ducts.

Treatment and outcome

Surgical treatment

Operative treatment is the best available therapy for cholangiocarcinoma. Not only may it cure a patient with early-stage disease (if the lesion can be located precisely), but it may also provide effective palliation for those with an irresectable tumour. Whenever possible, tumour resection should be performed unless there is clear evidence of advanced disease. A series of 29 non-jaundiced patients with opisthorchiasis and peripheral-type cholangiocarcinoma who underwent liver resection has been reported[41]. Twenty-one patients had tumour only in the right lobe, seven had a tumour in the left lobe, and one had tumour involving both lobes. Operations included right hepatectomy (19), extended right hepatectomy (four), left hepatectomy (two), left lobectomy (two) and medial segmentectomy (two). Fourteen patients had died by the end of the first postoperative year, five by the end of the second year, and a further five by the end of the third year. Five patients survived until the end of the fifth year, resulting in an actual 5-year survival rate of 17 per cent.

In a series of 30 patients with hilar cholangiocarcinoma associated with *O. verrini* infestation, the criteria for diagnosis of opisthorchiasis were (1) a history of previous positive stool examination for *Opisthorchis* or its eggs, (2) identification of *Opisthorchis* or its eggs in the stool or bile, (3) demonstration of a typical bead-like cholangiogram, and (4) histological evidence of the fluke in the specimen[43]. Seven patients had their tumours removed, four with concomitant liver resection. The four patients who underwent hepatectomy were anicteric before operation. Twenty-two patients underwent palliative biliary bypass procedures to a segmental duct (segment III duct in 18 patients and another segmental duct in four). For those undergoing resectional procedures, the actual 1-year survival rate was 86 per cent and the 2-year survival rate was 43 per cent. No patient survived until the end of the third year. The mean survival of patients undergoing palliative biliary bypass procedures was 8 months and the 1-year survival rate was only 26 per cent. All patients died within 2 years. Some jaundiced patients in this series were found to have several abnormal autonomic function test results[69], although the significance of these findings needs further investigation.

Generally, non-jaundiced patients with opisthorchiasis seem to have a better long-term outcome after operation than those who are icteric. Patients with a peripheral-type cholangiocarcinoma have a better prognosis than those with a hilar tumour; similar findings occur in patients with cholangiocarcinoma without associated liver fluke infestation[70]. Successful curative tumour resection is uncommon, although in approximately one-quarter of the patients the tumour can be resected[43]. The fact that up to three-quarters of the patients already have lymphatic or vascular invasion, or peritoneal seedings, at the time of operation makes aggressive surgery unlikely to be beneficial. By and large, survival after operation for cholangiocarcinoma associated with opisthorchiasis is similar to that in patients without liver fluke infestation[70,71]. Although there are no data available for the long-term outcome following surgical treatment of *C. sinensis*- or *O. felineus*-associated cholangiocarcinoma, survival can be expected to be more or less similar to that of the patients with opisthorchiasis described above.

Chemotherapy and immunomodulation

As periductal fibrosis and thickening of the bile duct walls, leading to bile duct obstruction, are consistent pathological findings, and intraluminal obstruction by flukes is frequently seen in the liver of patients with liver fluke infestation regardless of the development of cholangiocarcinoma[35], there is no significant role for available adjuvant chemotherapy after operation because of the risk of cholangitis and sepsis[41,43]. Progress in identifying suitable or new forms of chemotherapeutic agents is slow because this tumour is rare in the developed countries where most research institutes and pharmaceutical companies are located. However, several anticancer drugs, especially from plant products, have been tested against liver fluke-associated cholangiocarcinoma in Thailand[72,73]. Triptolide, a diterpene from *Tripterygium wilfordii*, has been shown to be highly effective against cholangiocarcinoma by *in vitro* cytotoxicity assay and *in vivo* inhibition of tumour growth in hamsters. It can induce apoptosis in several cholangiocarcinoma cell lines, and the appearance of apoptosis and DNA fragmentation occurs within 24 h of exposure to the agent[73]. The fact that the ED_{50} (effective dose that inhibits 50 per cent of cell growth) of triptolide when tested against these cell lines is lower than that of other antitumour compounds suggests that it may have therapeutic potential for development into an effective drug against this form of bile duct cancer[72–74].

More recently, tumour necrosis factor (TNF) α, at a concentration of 760 and 100 pg/ml in the presence of actinomycin D 1 μg/ml, has induced 50 per cent cell death in the two established human cholangiocarcinoma cell lines. The death of these two cell lines has been shown to be through apoptosis, and TNF-α type I receptor is involved in the process by generating sufficient signal to activate caspase group II enzymes, which are key enzymes in initiating the apoptotic event[75]. Pharmacological modulation of this group of enzymes may have a role in the treatment of cholangiocarcinoma in the future.

Conclusion

Although the association between liver fluke and cholangiocarcinoma has been recognized for more than half a century, and the carcinogenesis induced by the fluke has been actively investigated, the actual causal relationship between the parasite and the cancer is still poorly understood. The molecular biology of the tumour must be further studied. Identification of a marker for the tumour at an early stage is essential if the outcome of surgery is to be improved. Similarly, improvement in imaging technology to locate the tumour precisely will be important. New forms of treatment in addition to surgical resection, as well as effective means of controlling liver flukes, should also be explored.

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Systematic review and meta-analysis of interventions for postoperative fatigue

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Background: Postoperative fatigue is common, even after uncomplicated operations. Various theories have been presented regarding its aetiology, each suggesting different possible interventions. The purpose of this review was to identify all studies that have assessed interventions for postoperative fatigue and to evaluate these interventions using meta-analytical techniques.

Methods: Randomized controlled trials of interventions, identified from a systematic search of relevant databases, were evaluated according to standardized criteria and categorized according to intervention modality. Data relating to the efficacy of each intervention at four different postoperative time-points were collated and data synthesis by meta-analysis was performed.

Results: Analgesia is effective in reducing fatigue immediately after operation. Perioperative administration of human growth hormone reduces fatigue between 8 and 30 days after abdominal surgery. Weaker evidence was found to suggest an influence of glucocorticoid administration and of surgical technique on fatigue in the first week after operation. No evidence was found to support the theory that psychosocial or nutritional interventions affect the symptom.

Conclusion: While the results demonstrate that improved analgesia can attenuate immediate postoperative fatigue in most patient groups, further research is needed to determine whether the efficacy of human growth hormone and glucocorticoids extends beyond abdominal surgery. The paucity of research into cognitive–behavioural, sleep and activity-based interventions also needs to be addressed.

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Introduction

Postoperative fatigue is the principal subjective problem facing many patients following otherwise uncomplicated surgery[1]. For those affected, the symptom manifests itself as 'an indefinable weakness throughout the body after doing only minor tasks' requiring 'the [frequent] necessity of sitting or lying down …[and] an increased need for sleep'[2]. Postoperative fatigue may have major implications for a patient's subsequent recovery[3–5].

Unfortunately, the aetiology of the symptom remains poorly understood. The most commonly cited explanation is that summarised by Christensen[6], who suggested that the catabolic effects of the surgical stress response, combined with impaired postoperative nutritional intake and decreased mobility, contribute to fatigue by reducing skeletal muscle mass. Impaired cardiorespiratory functioning may also be implicated[7]. Encouraging anabolism through improved nutrition[8,9] or administration of human growth hormone (HGH)[10–13], and reducing the surgical stress response by means of minimally invasive surgery[14,15], spinal blockade[16] or glucocorticoid administration[17–19], have all been suggested as possible interventions.

Alternative theories have variously emphasized subclinical mineral deficiencies[20], increased brain tryptophan concentrations[21], disrupted cytokine production[22,23], disturbed sleep patterns[24,25] and self-fulfilling patient expectations coupled with negative mood[26] as aetiological factors. Clearly, each of these theories carries its own unique implications for potential intervention.

A systematic review was conducted to identify all randomized controlled trials (RCTs) that have assessed the impact of different interventions on postoperative fatigue, and to determine, using meta-analyses where appropriate, whether any of these interventions is effective.

Methods

Search strategy for the identification of studies

Two database searches were conducted. An initial search was performed on Medline, Embase, CINAHL, PsychInfo, ISI Science, ISI Social Science, ISI Index to Scientific and Technical Proceedings, ASSIA, PreMedline, the Cochrane Collaboration Library, HealthSTAR and the Index to Theses, all of which were examined from inception to July 2001 for English-language papers containing surgery-related MeSH or free-text terms in combination with fatigue-related MeSH or free-text terms. The full strategy used is available from the authors.

A second search was subsequently made to identify additional surgical studies that utilized the profile of mood states (POMS) questionnaire[27], a 65-item questionnaire used by several studies identified in the initial search, and which contains a reliable seven-item fatigue subscale together with scales designed to assess, for example, anger and depression. This search was performed using the same databases and surgical search terms as before, but substituting 'POMS or profile of mood states' for the fatigue-related terms.

The citations of all papers included in the review were examined for additional studies, as were the reference sections of 88 reviews, editorials and commentaries identified during the review process. Finally, an independent unpublished systematic search for postoperative fatigue-related papers[28] was examined to check the comprehensiveness of the strategy. This search, performed on a limited number of databases for the period 1966–1999, identified 16 relevant RCTs.

Inclusion criteria

Only RCTs reported in English were eligible for inclusion in the review, with an RCT defined as a study in which patients were allocated to treatment groups on the basis of a random or quasi-random method (e.g. using random number tables, hospital number, date of birth).

As the primary outcome for the review was subjective fatigue following surgery, only studies that used self-report fatigue measures after operation were eligible for inclusion. A fatigue measure was defined as any attempt to quantify the intensity of subjective fatigue, excluding, for example, single yes/no items. Eligible studies were also required to have made at least one fatigue assessment more than 24 h after operation.

Studies were excluded if not all the participants in the sample had undergone operation, if any of the participants had undergone fatigue-related adjuvant therapy or operation (e.g. chemotherapy, radiotherapy, bilateral adrenalectomy), or if any of the participants had a fatigue-related diagnosis (e.g. chronic fatigue syndrome, fibromyalgia, pregnancy, univentricular heart). Owing to the high frequency of chemotherapy, radiotherapy and immunotherapy used in breast cancer treatment and organ transplantation, all studies specifically examining these procedures were excluded.

Quality assessment

The methodological quality of included studies was assessed on four scales relating to the fatigue questionnaire (2, validity and reliability has been demonstrated; 0, validity and reliability not demonstrated), blinding (2, double blind; 1, single blind; 0, no blinding), concealment of allocation at enrolment (2, truly random allocation; 1, semi-secure randomization; 0, non-secure randomization) and completeness of follow-up (2, 100 per cent follow-up or intention to treat analysis; 1, 80–99 per cent follow-up; 0, follow-up 79 per cent or less). Where insufficient information was presented to assess an aspect of methodological quality, no points were given. A total quality score of between zero (worst quality) and eight (best quality) was then allocated to each RCT.

Data analysis

Data were pooled within studies to give no more than one outcome at each of the following time-points: same day as surgery to postoperative day 1 (d0–d1), postoperative day 2 to day 7 (d2–d7), postoperative day 8 to day 30 (d8–d30) and postoperative day 31 or later (d31+). Meta-analyses were then conducted at each time-point under a random-effects model, using MetaView version 4.1 for Windows (Update Software, Oxford, UK)[29] to examine the efficacy of various interventions compared with routine care or placebo. Where both routine care and placebo groups were used in a study, the placebo group was taken as the reference category.

To enable aggregation of results from RCTs using different fatigue scales, the difference between outcomes for the treatment and no-treatment groups within each study was first divided by the pooled standard deviation for these outcomes. The overall results are therefore presented as the standardized mean difference (SMD) with 95 per cent confidence intervals (c.i.), with a negative SMD indicating a lower fatigue score in the intervention group. Heterogeneity was assessed with Q tests, with $P < 0{\cdot}1$ taken as indicating significant heterogeneity to allow for the low power of this technique.

Review process

The study selection, quality assessment, data extraction and analyses were performed by one reviewer (G.J.R.), with uncertainties resolved through consultation with the second author (M.H.).

Results

Search results

The searches retrieved a total of 5325 references, of which 648 were examined in full. Five hundred and eighty (90 per cent) of these were subsequently excluded for: having no, or an inadequate, fatigue measure (197 papers), using an inappropriate design (187), using an inappropriate sample (103), being a review paper (88) or not being reported in English (five). Sixty-eight papers providing data on 66 RCTs were included. Fifty of these were identified through the initial search and 16 from the POMS-specific search. Cross-checking with the independently performed systematic search[28] did not reveal any missed references.

The intervention modalities examined by the 66 RCTs were analgesia (17 studies; n = 1084)[16,30–44], psychosocial (16 studies; n = 1546)[45–61], nutritional (eight studies; n = 258)[8,9,62–65], operative technique (six studies; n = 228)[14,15,66–69], anaesthesia (six studies; n = 324)[70–75], HGH (four studies; n = 138)[10–13,76], glucocorticoids (three studies; n = 64)[17–19] and other interventions (six studies; n = 785)[28,77–81].

Analgesia interventions

Studies included in this category (*Table 1*) investigated a diverse range of interventions, including transcutaneous electrical nerve stimulation (TENS)[34–37] (four studies), epidurals[16,30,31,33] (five studies), analgesia via interpleural catheter[32] (one study), self-administered *versus* nurse-administered analgesia[41,43] (two studies), fentanyl *versus* morphine[38] (one study), hydromorphone *versus* morphine[40] (one study), supplemental morphine at night-time[39] (one study), ketorolac *versus* placebo[42,44] (two studies) and ibuprofen *versus* placebo[42] (one study). These interventions were conducted on abdominal[16,31–33,35,40,41], gynaecological[34,36,37,39,42,44], orthopaedic[30,38] and cardiac[43] patients.

Eleven comparisons between increased analgesia and routine care or placebo provided sufficient information for

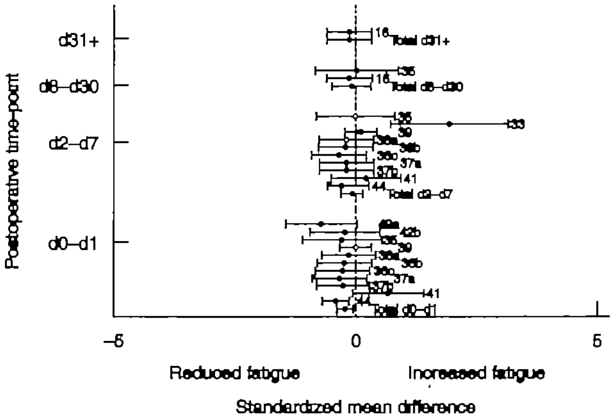

Fig. 1 Effect of analgesia on postoperative fatigue. Bars indicate 95 per cent confidence intervals

inclusion in a meta-analysis for d0–d1, with ten identified at d2–d7, two at d8–d30 and one at d31+. As illustrated in *Fig. 1*, increased analgesia was found to reduce postoperative fatigue significantly at d0–d1, with a SMD of – 0·21 (95 per cent c.i. – 0·37 to – 0·04; P = 0·01). Fatigue at the other time-points was not significantly affected, with SMDs of – 0·06 (– 0·29 to 0·16; P = 0·6), – 0·08 (– 0·48 to 0·32; P = 0·7) and – 0·12 (– 0·58 to 0·34; P = 0·6) respectively. Of the five comparisons not included in the analyses but for which results were available, none showed any effect of increased analgesia at any time-point[30–32,34].

Perhaps surprisingly, given the differing nature of the interventions included in these analyses, little heterogeneity was observed at d0–d1 (Q = 11·21, 10 d.f., P = 0·34) or at d8–d30 (Q = 0·10, 1 d.f., P = 0·75). Significant heterogeneity was identified at d2–d7, however (Q = 16·19, 9 d.f., P = 0·06). To examine whether this heterogeneity was due to a difference between opioid and non-opioid interventions, subgroup analyses were performed for opioid-related RCTs[33,39,41,44] and TENS-related RCTs[35–37]. Significant heterogeneity was still present for the opioid group (Q = 14·60, 3 d.f., P < 0·01) but not for the TENS group (Q = 0·49, 5 d.f., P = 0·99), suggesting that this distinction was not the sole source of heterogeneity. An alternative subgroup analysis comparing interventions shown to provide high pain relief *versus* those that gave low pain relief was not feasible as the main outlier that contributed most to the heterogeneity[33] failed to provide the required information.

Psychosocial interventions

A variety of psychosocial interventions was identified (*Table 2*), including emotionally disclosive writing[45] (one

Table 1 Randomized clinical trials of analgesia

Reference	Sample	Intervention*	Findings	Quality
16	Major abdominal surgery	Epidural (38) *versus* conventional analgesia (34)	No significant difference preop. or at d10, d20 or d30 ($P > 0{\cdot}05$)	3
30	Knee arthroplasty	Epidural (10) *versus* conventional analgesia (10)	No significant differences in fatigue preop., d1, d2, d3, d4, d5, d6 or d7 ($P > 0{\cdot}05$). No mean fatigue data reported	1
30	Hip arthroplasty	Epidural (11) *versus* conventional analgesia (11)	No significant differences in fatigue preop., d1, d2, d3, d4, d5, d6 or d7 ($P > 0{\cdot}05$). No mean fatigue data reported	1
31	Open cholecystectomy	Epidural and indomethacin (12) *versus* conventional analgesia (12)	No significant differences in change in fatigue from preop. to d1, d2, d4 or d8 ($P > 0{\cdot}05$). No mean fatigue data reported	3
32	Open cholecystectomy	Interpleural catheter (8) *versus* narcotic infusion (8)	No significant differences preop. or at d1, d2, d3, d7, d14, d28 or d90. No mean fatigue data reported	2
33	Abdominal surgery	Epidural fentanyl (8) *versus* intramuscular morphine (9)	No significant difference preop. ($P > 0{\cdot}05$). Increased fatigue in epidural group at d3 ($P < 0{\cdot}01$)	2
34	Lower abdominal gynaecological surgery	Sham TENS (25) *versus* TENS at non-acupoints (25) *versus* TENS at incision (25) *versus* TENS at acupoints (25)	No results reported preop. or at d1, d2 and d3. Presumably no significant differences	1
35	Retroperitoneal lymph node dissection	Acupuncture and transcutaneous stimulation (12) *versus* routine care (12)	No significant differences preop. or at d1, d2, d3, d5, d10, d15 or d20	2
36	Lower abdominal gynaecological surgery	Sham TENS (25) *versus* 2-Hz TENS (25) *versus* 100-Hz TENS (25) *versus* 2–100-Hz alternating TENS (25)	No significant differences preop., d1 or d2	3
37	Lower abdominal gynaecological surgery	Routine care (26) *versus* sham TENS at acupoints (25) *versus* low-intensity TENS at acupoints (25) *versus* high-intensity TENS at acupoints (25)	No significant differences preop. or at d1 or d2	3
38	Hip and knee arthroplasty	Fentanyl PCA (24) *versus* morphine PCA (23)	No significant difference preop. or at d3 ($P > 0{\cdot}05$). Significantly lower fatigue in fentanyl group at d1 and d2 ($P < 0{\cdot}05$)	7
39	Abdominal hysterectomy	PCA with night-time morphine infusion (74) *versus* conventional PCA (74)	No significant differences at d0, d1 or d2 ($P > 0{\cdot}05$)	1
40	Lower abdominal or pelvic surgery	Hydromorphone PCA (31) *versus* morphine PCA (30)	No significant difference in change in fatigue from preop. to d1 ($P > 0{\cdot}05$)	7
41	Abdominal surgery	PCA (17) *versus* nurse-administered analgesia (14)	No significant difference at d0 (8 h postop.), d1 (morning, mid-day and evening) or d2 (morning and mid-day) ($P > 0{\cdot}05$). Significantly more fatigue in PCA group at d0 (4 h postop.) and d1 (afternoon) ($P < 0{\cdot}05$)	3
42	Tubal ligation	Oral placebo and intravenous ketorolac (15) *versus* oral ibuprofen and intravenous placebo (15) *versus* oral placebo and intravenous placebo (15)	Significance between groups not reported	4
43	Cardiac	Nurse-administered oral analgesia (28) *versus* self-administered oral analgesia (30)	No mean fatigue data reported	3
44	Abdominal hysterectomy	Ketorolac (98) *versus* placebo (100)	No significant differences at d0 (day and evening), d1 (morning, evening and night) or d3 (morning and evening) ($P > 0{\cdot}05$). Significantly reduced fatigue in ketorolac group at d0 (night) ($P < 0{\cdot}05$)	3

*Values in parentheses are numbers of patients. d, Day; TENS, transcutaneous electrical nerve stimulation; PCA, patient-controlled analgesia study), emotional or psychiatric support[46,51] (two studies), relaxation training[47,49,50,54] (four studies), coping skills training[48,51–53,57] (five studies) and provision of information[52,53,55,56,58–61] (seven studies). The majority of these studies were conducted using patients undergoing cardiac surgery[47–52,57–60], although those having orthopaedic[45,54], gynaecological[53,55,56], malignant melanoma[46] and day surgery[61] were also represented.

No comparisons were available on the efficacy of psychosocial interventions at d0–d1, although four were available at both d2–d7 and d8–d30, and three at d31+. No significant effect was apparent at any of these points, with a SMD of $-0{\cdot}13$ (95 per cent c.i. $-0{\cdot}48$ to $0{\cdot}23$; $P = 0{\cdot}50$), $-0{\cdot}16$ ($-0{\cdot}37$ to $0{\cdot}04$; $P = 0{\cdot}12$) and $-0{\cdot}27$ ($-0{\cdot}67$ to $0{\cdot}13$; $P = 0{\cdot}19$) respectively (*Fig. 2*). Despite the *prima facie* heterogeneity of the treatments, no statistically significant

Table 2 Randomized clinical trials of psychosocial interventions

Reference	Sample	Intervention*	Findings	Quality
45	Knee arthroscopy and arthroplasty	Postoperative disclosive writing about the injury (15) *versus* writing about diet (15)	No main effect of intervention ($P > 0{\cdot}05$). No mean fatigue data reported	4
46	Surgery for malignant melanoma	Postoperative psychiatric support group (38) *versus* routine care (28)	No difference preintervention or 42 days postintervention ($P > 0{\cdot}05$). Significant reduction in fatigue 6 months postintervention ($P < 0{\cdot}05$)	2
47	CABG	Self-hypnosis and relaxation training (13) *versus* routine care (9)	No significant difference in fatigue change scores (preop. to d5; $P > 0{\cdot}05$)	2
48	Cardiac surgery	Family coping and conflict resolution training (72) *versus* routine care (77)	No mean fatigue data reported. Number of patients estimated from overall drop-out rate	5
49	Cardiac surgery	Relaxation training (13) *versus* routine care (14)	No significant difference in fatigue change scores (preop. to discharge; $P > 0{\cdot}05$)	3
50	CABG	Guided imagery training (47) *versus* routine care (47)	Reported reduced fatigue in imagery group (d0–d7). No mean fatigue data reported	1
51	CABG	Coping skills training (19) *versus* emotional support (20) *versus* routine care (19)	No significant effect ($P > 0{\cdot}05$). No fatigue data reported for separate study arms. Number of patients estimated from overall drop-out rate	3
52	CABG	Cognitive coping skills booklet (26) *versus* sensory and procedural information booklet (26) *versus* minimally informative booklet (26) *versus* routine care (46)	No significant differences at preop., d5, d14 or d49 ($P > 0{\cdot}05$). Significantly less fatigue at d28 compared with routine care for sensory and procedural information group ($P < 0{\cdot}05$) and for minimally informative booklet and cognitive coping booklet groups ($P < 0{\cdot}01$)	4
53	Abdominal hysterectomy	Cognitive coping (20) *versus* sensory information (20) *versus* minimally informative information (20)	No significant differences at preop., d3 or d24 ($P > 0{\cdot}05$). No mean fatigue data reported	3
54	Hip and knee arthroplasty	Imagery group (53) *versus* relaxation group (53) *versus* control group (54)	No effect at d1, d3, d7, d30 or 6 months. Limited methodological information available and no fatigue data presented. Numbers of patients estimated from overall sample size	2
55 and 56	Minor gynaecological surgery	Maximally informative booklet (20) *versus* minimally informative booklet (17) *versus* routine care (26)	No differences preop. or at d7 or d42 ($P > 0{\cdot}05$)	4
57	Cardiac	Basic education and counselling (35) *versus* basic education, counselling and follow-up reinforcement and monitoring (32)	No mean fatigue data reported	4
58	CABG	Preop. educational visit to hospital (20) *versus* routine care (20)	No mean fatigue data reported	2
59	CABG	Procedural and sensory information (38) *versus* routine care (44)	No significant difference at d28 ($P > 0{\cdot}05$)	3
60	CABG	Postoperative sensory information about recovery (90) *versus* routine care (90)	No significant difference preintervention or at d30 ($P > 0{\cdot}05$). No differences for male subgroup ($P > 0{\cdot}05$). Significant reduction in fatigue for female subgroup at d30 ($P < 0{\cdot}01$)	4
61	Day surgery	Detailed information (197) *versus* routine information (127)	No significant differences at d1, d2, d3 or d4 ($P > 0{\cdot}05$)	0

*Values in parentheses are numbers of patients. d, Day; CABG, coronary artery bypass graft heterogeneity was identified at any time-point ($Q = 3{\cdot}56$, 3 d.f., $P = 0{\cdot}31$; $Q = 0{\cdot}95$, 3 d.f., $P = 0{\cdot}81$; $Q = 3{\cdot}43$, 2 d.f., $P = 0{\cdot}18$). Of the five studies not included in these analyses that provided relevant results[45,50,51,53,54], only one[50] found a significant effect, with reduced fatigue reported during the first postoperative week for cardiac patients engaged in guided imagery.

Nutritional interventions

Two studies included in the review examined the effect of providing dietary advice and protein supplements to patients[8], two investigated immediate enteral feeding[62,63], one investigated early parenteral feeding[9], three examined the effect of providing general nutritional supplements[9,65] and one examined whether a specific glutamate supplement provided any benefit[64] (*Table 3*). Each of these studies used an abdominal or general surgical sample.

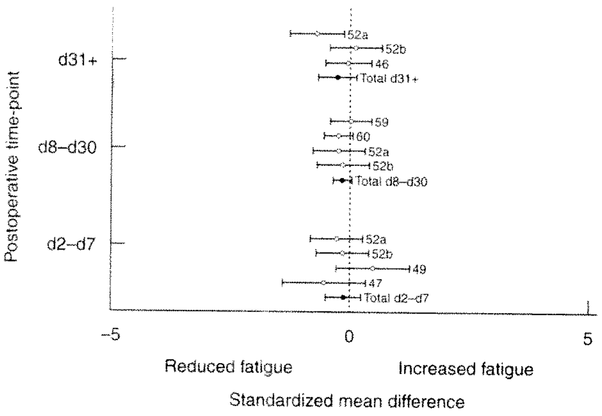

Fig. 2 Effect of psychosocial interventions on postoperative fatigue. Bars indicate 95 per cent confidence intervals

No study provided adequate data for use in a meta-analysis at d0–d1, with four, two and one studies providing data for d2–d7, d8–d30 and d31+ respectively (*Fig. 3*). The SMD at these time-points was $-0{\cdot}09$ (95 per cent c.i. $-0{\cdot}56$ to $0{\cdot}38$; $P = 0{\cdot}70$), $-0{\cdot}34$ ($-0{\cdot}90$ to $0{\cdot}23$; $P = 0{\cdot}20$) and $-0{\cdot}29$ ($-0{\cdot}99$ to $0{\cdot}40$; $P = 0{\cdot}40$), none reflecting a significant effect. No significant heterogeneity was identified at d2–d7 ($P = 0{\cdot}14$) or d8–d30 ($P = 0{\cdot}86$), with Q scores of 5·52 (3 d.f.) and 0·03 (1 d.f.) respectively. Three studies provided further relevant information that could not be entered into these analyses[8,9], none of which identified any significant effect of nutritional intervention.

Surgical technique

All studies except one in this category (*Table 4*) investigated the difference between laparoscopic and open surgery for the following procedures: colorectal resection[14], appendicectomy[15], colonic resection[66], Nissen fundoplication[67] and tubal ligation[68]. Clearly, the difference in invasiveness between laparoscopic and open surgery depends on the specific procedure under consideration, making it difficult to group the results of these trials. Therefore, no formal meta-analysis was attempted in this category.

Table 3 Randomized clinical trials of nutrition

Reference	Sample	Intervention*	Findings	Quality
8	Elective colorectal surgery	Dietary advice (17) *versus* routine care (15)	No significant differences preop. or at discharge, d20, d50 or d110. No mean fatigue data reported	4
8	Acute colorectal surgery	Dietary advice (9) *versus* routine care (12)	No significant differences preop. or at discharge, d20, d50 or d110. No mean fatigue data reported	4
9	Gastrointestinal surgery	Parenteral nutrition (8) *versus* routine care (6)	No significant differences preop. or at d14, d28, d42 or d84	4
9	General non-gastrointestinal surgery	Nutritional supplements (16) *versus* routine care (20)	No significant differences preop. or at d14, d28, d42 or d84. No mean fatigue data reported	5
62	Oesophagectomy	Immediate enteral feeding (13) *versus* routine care (15)	No significant differences preop. or at d6	4
63	Bowel resection or reanastomosis	Enteral nutrition (16) *versus* routine care (16)	No significant differences preop. or at d7, d14, d30 or d90	4
64	Open cholecystectomy	TPN plus glutamate supplement (9) *versus* TPN (8)	No significant differences preop. or at d3, d10, d20 or d30	3
65	Moderate to major gastrointestinal surgery	Nutritional supplements (39) *versus* routine care (39)	Significance between groups not reported	3

*Values in parentheses are numbers of patients. d, Day; TPN, total parenteral nutrition

Of these five studies, three[14,15,66] reported no significant difference at d0–d1, while one[67] reported reduced fatigue in the laparoscopic group at this time. Three studies[15,66,68] reported no significant difference at d2–d7, while two[14,67] reported less fatigue in the laparoscopic group. None of the three studies that provided data reported a significant difference between the groups at d8–d30[15,66,67]. No data were available for d31+. No significant difference was identified at d0, d1 or d2 by the final surgical technique study, which compared French and American styles of laparoscopic cholecystectomy[69].

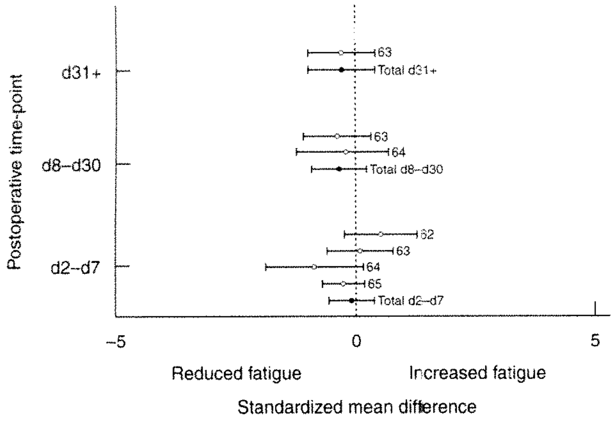

Fig. 3 Effect of nutritional interventions on postoperative fatigue. Bars indicate 95 per cent confidence intervals

Anaesthesia

The anaesthesia studies included in the review examined the effects of qualitatively different techniques (*Table 5*), prohibiting the use of meta-analysis. While no differences were identified between different isomers of ketamine[71] or between isoflurane and propofol[73], significantly higher fatigue levels were reported at d0–d1 for 200 mg pethidine compared with 100 or 50 mg pethidine, or 250 mg prilocaine[70], and for thiopentone compared with propofol at d0–d1[72]. Significantly greater fatigue was also reported for propofol compared with desflurane at d7, but not at d0 or d1[74].

Human growth hormone

Three studies were identified that compared HGH with placebo following major abdominal surgery[10–12] (*Table 6*). An additional study[76] compared administration of Cetornan® nutritional supplement (Laboratoires J Logeais, Paris, France), reported to stimulate HGH secretion, with a formula peptide supplement following surgery for hip fracture.

For the meta-analyses, none, three, two and three comparisons were available for inclusion at the four respective time-points (*Fig. 4*). The SMD was found to be

Table 4 Randomized clinical trials examining surgical technique

Reference	Sample	Intervention*	Findings	Quality
14	Colorectal resection	Laparoscopic (30) *versus* open (30)	No significant difference at d1 ($P > 0.05$). Significantly less fatigue in laparoscopic group at d2, d3, d4, d5, d6 and d7. No mean fatigue data reported	2
15	Appendicectomy	Laparoscopic (18) *versus* open (21)	No significant differences at d0, d1, d2, d3, d4, d5, d6, d7, d8–d21 ($P > 0.05$). No mean fatigue data reported	2
66	Colonic resection	Laparoscopic (15) *versus* open (14)	No significant differences preop. or at d0, d1, d2, d3, d4, d5, d10 or d30. No mean fatigue data reported	5
67	Nissen fundoplication	Laparoscopic (21) *versus* open (20)	No significant differences on any day from d8 to d21 ($P > 0.05$). Significantly less fatigue in laparoscopic group at d0, d1, d2, d3, d4, d5, d6 and d7 ($P < 0.01$). No mean fatigue data reported	3
68	Tubal ligation	Minimally invasive surgery under local anaesthetic (7) *versus* open surgery under general anaesthetic (7)	No significant difference at d7	2
69	Laparoscopic cholecystectomy	French technique (23) *versus* American technique (22)	No significant difference at d0, d1 or d2 ($P > 0.05$)	4

*Values in parentheses are numbers of patients. d, Day

−0·33 (95 per cent c.i. −0·74 to 0·08; $P = 0{\cdot}11$) at d2–d7, −0·55 (− 1·13 to −0·17; $P = 0{\cdot}01$) at d8–d30 and −0·39 (− 1·58 to 0·80; $P = 0{\cdot}50$) at d30+, suggesting a significant beneficial effect of HGH treatment only at d8–d30. Significant heterogeneity was identified at d31+ ($Q = 11{\cdot}21$, 2 d.f., $P < 0{\cdot}01$), but not at d2–d7 ($Q = 0{\cdot}68$, 2 d.f., $P = 0{\cdot}71$) or at d8–d30 ($Q = 0{\cdot}75$, 1 d.f., $P = 0{\cdot}39$). Given the limited amount of data available at d31+, no statistical attempt was made to identify the source of the heterogeneity at this point. The Cetoran study[76], while providing insufficient data for inclusion in the meta-analysis, reported that the intervention significantly reduced fatigue at day 56.

Glucocorticoids

Three studies examined the effect of perioperative glucocorticoid administration in patients undergoing abdominal

Table 5 Randomized clinical trials of anaesthesia

Reference	Sample	Intervention*	Findings	Quality
70	Arthroscopic examination and/or surgery	200 mg pethidine (10) *versus* 100 mg pethidine (10) *versus* 50 mg pethidine (10) *versus* prilocaine local anaesthesia (10)	No significant difference preop. or at d0 (200 min, 4 h, 5 h, 6 h and 12 h). Significantly greater fatigue in 200-mg pethidine group compared with prilocaine group at d0 (60 min; $P < 0{\cdot}05$). Significantly greater fatigue in 200-mg pethidine group compared with other three groups at d0 (80 and 140 min; $P < 0{\cdot}05$)	6
71	Minor outpatient surgery	With ketamine isomer (21) *versus* without ketamine isomer (19) *versus* racemic ketamine (20)	No significant differences at 1–2 h or d1 ($P > 0{\cdot}05$). No mean fatigue data reported	5
72	Day surgery dilatation and curettage	Thiopentone (30) *versus* propofol (30)	No significant differences immediately after operation or at 2 h ($P > 0{\cdot}05$). Significantly less fatigue in propofol group at 1 h ($P < 0{\cdot}01$) and d1 ($P < 0{\cdot}05$). Numbers of patients estimated from overall sample size	2
73	CABG	Isoflurane (24) *versus* propofol (24)	No significant difference preop. or at discharge ($P > 0{\cdot}05$)	5
74	Laparoscopic cholecystectomy	Desflurane (27) *versus* propofol (29)	No significant differences at d0 or d1 ($P > 0{\cdot}05$). Significantly less fatigue in desflurane group at d7 ($P < 0{\cdot}01$)	3
75	Major abdominal surgery	Isoflurane + fentanyl + nitrous oxide (20) *versus* propofol + fentanyl + nitrous oxide (20) *versus* propofol + fentanyl (20)	Fatigue results combined with those for lack of appetite and dizziness to give a measure of neurasthenia	0

*Values in parentheses are numbers of patients. d, Day; CABG, coronary artery bypass graft

Table 6 Randomized clinical trials of human growth hormone

Reference	Sample	Intervention*	Findings	Quality
10	Ileoanal J pouch surgery	HGH 2 days preop. to 7 days postop. (12) *versus* placebo preop. and postop. (12)	No significant differences preop. or at d10 or d30 ($P > 0{\cdot}05$). Significantly less fatigue in HGH group at d90 ($P < 0{\cdot}01$)	6
11	Abdominal aortic aneurysm repair	rHGH for 6 days preop. and postop. (10) *versus* placebo for 6 days preop. and rHGH for 6 days postop. (11) *versus* placebo preop. and postop. (10)	No significant differences in fatigue at 7 days preop., 1 day preop., d7 or d60 ($P > 0{\cdot}05$)	4
12	Open cholecystectomy	Parenteral nutrition and HGH from 2 days preop. to 5 days postop (22) *versus* parenteral nutrition and placebo (26)	Significance levels between groups not reported	5
76	Surgery for hip fracture	Cetornan® nutritional supplement (17) *versus* formula peptide nutritional supplement (18)	Cetoran used to stimulate HGH and insulin secretion. Cetoran group had significantly lower fatigue at d56. No mean fatigue data reported	2

*Values in parentheses are numbers of patients. d, Day; HGH, human growth hormone; rHGH, recombinant human growth hormone

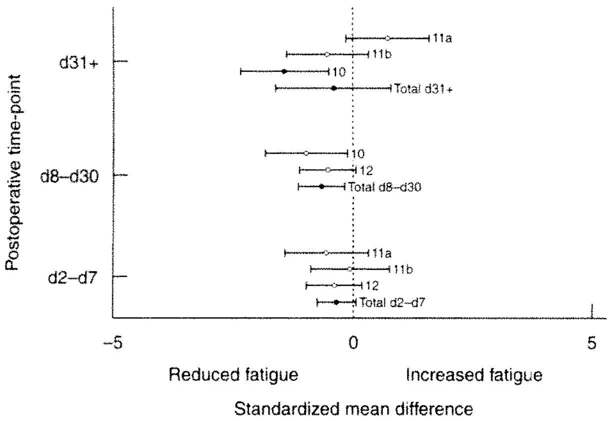

Fig. 4 Effect of human growth hormone treatment on postoperative fatigue. Bars indicate 95 per cent confidence intervals

surgery[17–19] (*Table 7*). Although insufficient data were available to warrant a meta-analysis, one study[17] reported a significant beneficial effect of glucocorticoids at d0–d1, but not during d2–d7. A second study[19] reported no significant difference between glucocorticoid and placebo groups at d0–d1, d2–d7 or d8–d30, and the final study[18] reported an overall beneficial effect of the glucocorticoid provision over d1, d2, d4 and d8.

Other interventions

Details of the other types of intervention included in the review are provided in *Table 8*. It can be seen that limited chair-nursing following lower-limb surgery[28], increased perioperative hydration during dental surgery[79], and the administration of Effortil® (Boehringer Ingelheim,

Table 7 Randomized clinical trials of glucocorticoids

Reference	Sample	Intervention*	Findings	Quality
17	Abdominal surgery	Perioperative methylprednisolone (10) *versus* placebo (10)	No significant differences preop. or at d2, d3, d4, d5, d6 or d7 ($P > 0.01$). Significantly less fatigue in methylprednisolone group at d1 ($P < 0.05$)	6
18	Colonic resection	Perioperative methylprednisolone and neural blockade with postoperative hydrochloride-morphine and indomethacin sodium (11) *versus* routine care (9)	Overall (d1, d2, d4 and d8) change from preoperative fatigue scores were significantly lower in methylprednisolone group ($P < 0.05$). No mean fatigue data reported	3
19	Colonic resection	Perioperative methylprednisolone (12) *versus* placebo (12)	No significant difference in change from preop. fatigue scores at d0, d1, d2, d3, d4, d5, d6, d7, d8 or d9 ($P > 0.05$). No mean fatigue data reported	7

*Values in parentheses are numbers of patients. d, Day

Table 8 Other randomized clinical trials

Reference	Sample	Intervention*	Findings	Quality
28	Lower-limb surgery	Limited chair nursing (100) *versus* unrestricted chair nursing (103)	No significant difference preop. or overall postop in mean fatigue (d2, d4, d6, d9, d13) ($P > 0.05$). Limited chair nursing significantly reduced risk of severe fatigue postop. ($P < 0.001$)	4
77	Inguinal hernia repair	Early mobilization (50) *versus* late mobilization (57)	No mean fatigue data reported	0
78	Laparoscopic cholecystectomy	Day surgery (14) *versus* overnight stay (14)	No significant difference overall (d1, d2, d3, d4)	0
79	Extraction of more than two molars	High-volume perioperative hydration (38) *versus* low-volume perioperative hydration (39)	No significant difference at d1 ($P > 0.05$). Significantly less fatigue at d0 (discharge and evening) ($P < 0.05$)	3
80	Major gynaecological surgery	Effortil (22) *versus* placebo (24)	No significant difference preop. or at d3, d4, d5, d6, d7 or d8 ($P > 0.05$). Significantly reduced fatigue in Effortil group at d1 and d2 ($P < 0.05$)	4
81	CABG	Lower haemoglobin threshold for transfusion (173) *versus* normal threshold (155)	No significant differences at d3 or d5 ($P > 0.05$). No mean fatigue data reported	4

*Values in parentheses are numbers of patients. d, Day; CABG, coronary artery bypass graft

Randburg, South Africa) after gynaecological surgery[80] have all been found to reduce postoperative fatigue significantly. Same-day discharge[78] following laparoscopic cholecystectomy and lowering the haemoglobin threshold for blood transfusion[81] for cardiac patients have not done so.

Discussion

Current evidence

The results of this review show that increased analgesia is effective in reducing fatigue during the immediate postoperative period. For patients having abdominal surgery there is also reasonable evidence that provision of perioperative HGH reduces fatigue in the d8–d30 period. More equivocal evidence supports the efficacy of glucocorticoids in such patients and the use of laparoscopic surgery for some procedures, such as Nissen fundoplication and colorectal resection. On the basis of the research to date, there is no evidence that psychosocial or nutritional interventions affect postoperative fatigue.

Research content

Unfortunately, interpretation of these findings is complicated to a degree by the different surgical populations included in the review. It has been reported, for example, that patients having major abdominal surgery experience greater fatigue than those undergoing minor procedures[82]. Similarly, orthopaedic procedures may also cause relatively little tiredness[3]. Whether interventions that failed to attenuate fatigue following, say, orthopaedic surgery would have been more successful in the major abdominal surgery setting is therefore a moot point. Until further research has been conducted it is difficult to draw any conclusions from these data about the efficacy of nutritional, HGH or glucocorticoid interventions in non-abdominal patients. It may be easier to extrapolate from the findings relating to analgesia and psychosocial interventions, given the range of surgical populations that have been included in the relevant studies.

It is also important to recognize that many of the RCTs included in this review were not designed specifically to reduce postoperative fatigue and provided little or no theoretical rationale as to why the intervention under study might be expected to attenuate it. This is particularly true of the psychosocial RCTs, 13 of which are reported here only because of their use of the POMS questionnaire to measure postoperative mood. That these interventions failed to demonstrate any significant effect on fatigue is relatively unsurprising, and it remains to be seen whether better targeted psychosocial interventions would prove more beneficial. Certainly, given the efficacy of cognitive–behavioural interventions in the treatment of other fatigue syndromes[83], the present authors believe that research along these lines might now also be indicated in this area.

Of those studies that did specifically target postoperative fatigue, it was notable that the large majority based their interventions solely on the catabolic surgical stress model[6] of the symptom. No studies were identified that attempted to reduce fatigue by improving postoperative sleep hygiene[25,84], decreasing the amount of plasma tryptophan available to cross the blood–brain barrier[21], or altering patient expectations or postoperative mood[26], as indicated by various alternative theories. Furthermore, despite the putative causal role of reduced mobility in causing postoperative fatigue[6], no RCTs were found that used physical activity-based interventions. There is thus scope for further research in each of these areas.

Research methodology

In general the quality of the research identified by this review was average to poor. Small sample sizes and incompletely reported methods and results were of concern, but more worrying still was the poor quality of the questionnaires used to assess postoperative fatigue. Attempts to assess fatigue objectively by measuring, for example, physical activity, time taken to return to normal routine, or involuntary or voluntary muscle force are problematic because they can be confounded by numerous factors including pain and anxiety. Much effort has therefore been devoted to developing short and easy-to-use questionnaires that provide some quantification of a patient's subjective feeling of fatigue. It was therefore disappointing to find that 23 studies utilized measures that have not had their validity or reliability evaluated empirically[14,15,30,34,36,37,39,42,44,50,61,67,68,70,72,74–80]. Future research should ensure that an adequate measure of subjective fatigue is employed, possibly in tandem with important objective measures, such as time taken to return to work.

Implications

In terms of the clinical implications of this review, the efficacy of perioperative HGH in reducing postoperative fatigue at d8–d30 is encouraging. As yet, however, this treatment has been examined in only a very small sample of patients undergoing abdominal surgery, and larger clinical trials are now needed to verify the finding and to examine the overall safety of HGH after operation before its use can be recommended in clinical practice. The efficacy of glucocorticoids in the immediate postoperative period may have less applicability, given the known immunosuppressive effects of these agents[17]. Improved analgesia may be more useful at this time-point.

In terms of theoretical implications, these findings suggest that postoperative fatigue may have a two-stage pathogenesis, with different aetiological factors active in early and late convalescence. The finding that interventions such as laparoscopic surgery and glucocorticoid provision appear to have most effect at d0–d1 and d2–d7 supports the suggestion that some acute aspect of the surgical stress response may play an important role during early recovery[23]. For example, both interventions dampen cytokine expression following surgery[19,66], a phenomenon that has recently been shown to correlate with fatigue[85]. As increased analgesia also reduces early fatigue, it is possible that sleep disturbance due to pain may also be a contributing factor[23,86]. Which variables are important during the later convalescent period is less clear. HGH, effective from day 8 to day 30, has variously been demonstrated to improve postoperative nitrogen balance, voluntary muscle function, pulmonary function and immunocompetence[10–13].

Conclusion

The results of this review suggest that postoperative fatigue immediately after operation may be attenuated by improved analgesia, perioperative administration of HGH and glucorticoids. However, many of the findings in this area require confirmation and extension to other surgical populations. There also remains much scope for novel interventions, not based on the surgical stress model of postoperative fatigue, to be attempted.

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Randomized clinical trial comparing self-expanding metallic stents with plastic endoprostheses in the palliation of oesophageal cancer

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Background: There is little evidence of the clinical and cost effectiveness of self-expanding metallic stents in the palliation of oesophageal cancer. The aims of this randomized trial were to evaluate the immediate and medium-term clinical outcomes following palliative intubation, examine patient quality of life, and evaluate costs and benefits from the perspective of the health service.

Methods: Fifty patients with inoperable oesophageal cancer were randomly allocated a metallic stent ($n = 25$) or plastic endoprosthesis ($n = 25$). Patients were followed up monthly until death.

Results: There was no significant difference in procedure-related complications or mortality rate between the two groups. There was a trend towards significance in favour of metallic stents with respect to quality of life and survival (median survival 62 *versus* 107 days for plastic prosthesis and metallic stent respectively). The cost of the initial placement of metallic stents was significantly higher than that of plastic endoprostheses (£983 *versus* £296). After 4 weeks, cost differences were no longer significant.

Conclusion: Metallic stents may contribute to improved survival and quality of life in patients with oesophageal cancer. Although initially more expensive, this cost difference does not last beyond 4 weeks. A larger trial involving approximately 300 patients would be required to detect a quality of life benefit of the magnitude observed in this trial.

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Introduction

Oesophageal cancer is increasing in the UK and has a poor outcome, with an overall 5-year survival rate of less than 10 per cent, and fewer than 50 per cent of patients are suitable for resection at presentation[1–3]. For most patients palliation is the only option.

Palliative treatment of the dysphagia resulting from progressive oesophageal cancer may include radiotherapy, chemotherapy, endoscopic dilatation, endoscopic ablation and the insertion of an endoprosthesis[1]. Originally, semirigid plastic endoprostheses were used. A more recent development is the self-expanding metallic stent[2]. These offer a lower insertion profile, do not generally require predilatation of the tumour and are inserted using only topical anaesthesia and conscious sedation. Once placed, they expand rapidly to a greater diameter than plastic endoprostheses.

To date, there have been five small randomized trials examining the clinical effectiveness of treatment with a metallic stent compared with a plastic endoprosthesis[4–8]. In general, these studies have shown that dysphagia initially improves for all patients. Procedure-related complications tended to be lower in patients with metallic stents and length of hospital stay was shorter. The 30-day mortality rate and overall survival were similar in both groups. In studies with an economic evaluation, the cost of initial placement of a metallic stent was greater[6,8]. No study, however, collected data on costs beyond the initial hospital admission. Only one study included any information on patients' quality of life, which was similar in both groups[6].

Studies are still required, therefore, that evaluate not only the clinical effectiveness of metallic stents compared with plastic endoprostheses but also their cost effectiveness beyond the initial placement period and impact on patients' quality of life. The aim of this study was to evaluate the

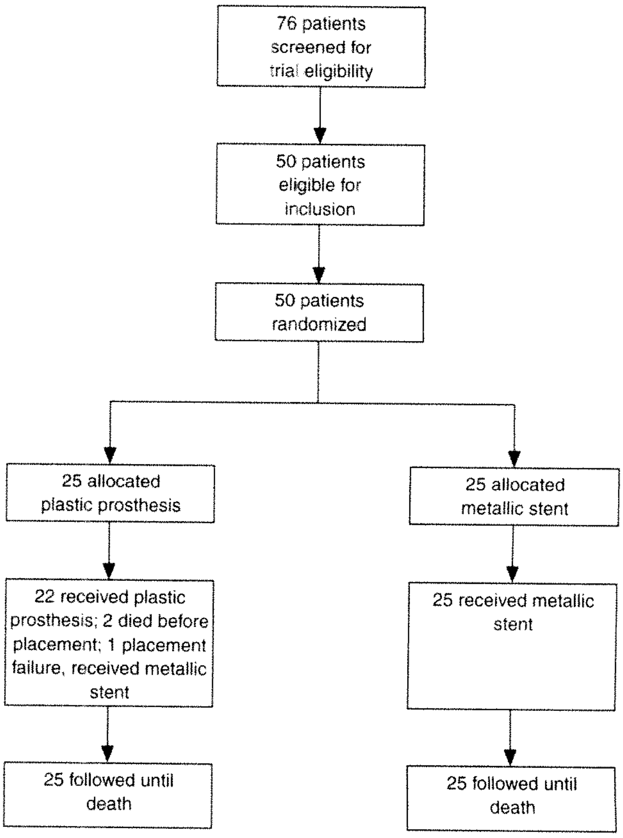
76 patients screened for trial eligibility
50 patients eligible for inclusion
50 patients randomized
25 allocated plastic prosthesis
25 allocated metallic stent
22 received plastic prosthesis; 2 died before placement; 1 placement failure, received metallic stent
25 received metallic stent
25 followed until death
25 followed until death

Fig. 1 Trial profile

immediate and medium-term outcomes following palliation, including procedure-related mortality, complications and reintervention rates. The study additionally aimed to examine quality of life for the patient before and after intervention, and evaluate the costs and benefits following intervention from the perspective of the health service.

At the time of designing this study, there was a lack of appropriate clinical data on which to base a sample size calculation. Therefore, this study was designed as a pilot to inform the design of a multicentre randomized controlled trial.

Patients and methods

Patients

Patients were eligible for inclusion in the study if they were over 35 years old and suffering from dysphagia due to a malignant tumour arising from the oesophagus, including the gastro-oesophageal junction, that was inoperable or if the patient was considered unsuitable for surgical resection owing to poor general health or advanced age. Previous treatment, e.g. laser or radiotherapy, did not exclude them from the trial and there were no restrictions on later adjuvant treatment, e.g. chemotherapy. Patients were excluded from the trial if there was no histological evidence of a tumour, a tracheo-oesophageal fistula or a tumour within 3 cm of the upper oesophageal sphincter.

Patients attending two acute hospital sites in Glasgow were assessed for eligibility by a research nurse. Of 76 patients assessed, informed written consent was obtained from 50 who met the inclusion criteria. After collection of baseline data, patients were randomized using a list of computer-generated numbers concealed in sealed envelopes to receive either a plastic endoprosthesis or metallic stent (*Fig. 1*). An individual (G.D.M.) not involved in the recruitment, treatment or evaluation of the patients oversaw the generation of this list. The study was approved by the local research ethics committee at each hospital site.

Placement of plastic endoprostheses

The Wilson Cook endoprosthesis (Wilson-Cook Medical, Winston-Salem, North Carolina, USA) was used throughout. This semirigid plastic endoprosthesis is reinforced with an inner core of stainless steel spiral coils, and has an inner diameter of 12 mm and an outer diameter of 17 mm. The appropriate stent length, ranging from 4·4 to 14·4 cm, was chosen after endoscopic assessment of tumour length. Predilatation (mean 15 (range 14–19) mm) was carried out in 18 patients. The prosthesis was delivered over a Savary–Gilliard dilator in which the overtube was inserted through the stricture by a pulsion technique with fluoroscopic guidance. Assessment of the prosthesis position was made using a combination of endoscopy and fluoroscopy. A consultant surgeon carried out the placements at each site. Conscious sedation (midazolam 2–10 mg) was used in most patients, although four required a general anaesthetic.

Placement of metallic stents

Covered self-expanding Wallstents™ (Boston Scientific, Watertown, Massachusetts, USA) were used in all but one patient who, owing to operator error, had a covered Ultraflex™ (Boston Scientific) stent placed. Two different Wallstent™ designs were used. The Flamingo™ (30 mm proximal flare and 20 mm distal flare, total length 140 mm) double-flared partly covered Wallstent™ was used in lower-third lesions and the distal end of the stent protruded unsupported into the stomach. The standard Telestep™ (25 mm diameter and 105 mm length) partly covered non-flared Wallstent™ was used in all other patients. Predilatation was carried out using a 12-mm balloon in six patients to overcome tumour resistance during stent placement or as a requirement of the covered Ultraflex™

stent. Balloon dilatation was not required following stent deployment. The procedure was carried out by two consultant radiologists or by specialist registrars under their direct supervision. Fluoroscopy was used exclusively for all placements. Conscious sedation was used in all patients (diamorphine 5 mg and midazolam 2–10 mg).

In both groups a water-soluble contrast swallow (Gastromiro™; Merck Pharmaceuticals, Ponders End, UK) was performed 24 h after placement of the prostheses to evaluate function, migration and exclude perforation. Plain radiography was carried out at 1 month to detect any further migration. The radiographs were reviewed by two independent consultant radiologists and any discrepancy resolved by consensus with one of the authors (J.G.M.). Patients in whom the stent or plastic endoprosthesis protruded across the cardia into the stomach were given prophylactic proton-pump inhibitors (omeprazole 20 mg per day) to minimize reflux symptoms.

Follow-up

Patients were evaluated immediately before treatment by the research nurse, then followed monthly at home until death. Dysphagia was measured on a five-point scale, from 0 (able to eat a normal diet) to 4 (complete dysphagia)[9]. The research nurse recorded complications, reinterventions, readmission and deaths prospectively.

Quality of life scores

Quality of life was measured before placement and afterwards at monthly intervals, in patients' homes, using the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 questionnaire plus an oesophageal cancer-specific module[10,11]. This multidimensional quality of life measure examines five functional scales, three symptom scales and a global health and quality of life scale. A high functional score and a low symptom score is favourable. On the functional scales death was scored as 0. Conversely, on symptom scales death was scored as 100. As survival was relatively limited, mean scores for the first 4 months following placement were used. The mean score for each patient was calculated after assigning the lowest possible functional scores (0) and the highest possible symptom scores (100) at visits that were missed because the patient had died.

Cost analysis

The analysis was undertaken from a health service perspective. Primary analysis was based on the cost of placing the initial prosthesis and subsequent reinterventions. Some patients had additional inpatient stays for reasons not directly related to the placement of a prosthesis, e.g. chemotherapy. These costs were not collected as part of the study; only the hospital inpatient stay based on routinely available data was included. This formed the basis of a secondary analysis.

The monetary valuation of resource use was undertaken by attaching unit costs at 1997–1999 prices. For disposable equipment, the unit cost per patient was the manufacturer's list price, including value added tax (VAT). For reusable equipment, the equivalent annual cost[12] was calculated using a 6 per cent discount rate[13] and estimates of each item's useful life expectancy. These costs were divided by the estimated annual throughput to give costs per patient. Drug costs were based on manufacturers' list prices including VAT[14]. Staff costs, including employers' costs, were based on the top point of the salary scale for consultants and on midpoint salaries for all other staff. Data on the number and type of staff involved in each procedure and the length of time taken for the procedure were collected prospectively and the total staff cost per procedure estimated.

Overhead costs associated with attendance in the radiology unit were obtained from routine data for the two sites[15]. No routine data were available for the costs of attending the endoscopy suite, so the radiological cost was used as a proxy. The cost of theatre use at one site was based on the total cost of a theatre hour. The length of the procedure was used to calculate the cost of theatre time per procedure. The contrast swallow was based on the routine cost of a radiography attendance, with staff costs calculated as before and using the manufacturer's list price for barium. Length of stay was calculated from the date of the initial placement to the date of discharge. The cost of an inpatient day was calculated from the routine cost of an inpatient week at both hospital sites[15]. The length of stay cost was then calculated, as appropriate. The cost of a day case was also based on routine data[15].

Statistical analysis

All analyses were carried out on an intention-to-treat basis. In accord with the pilot nature of this project, the majority of the analyses were descriptive. Formal statistical tests were based on non-parametric methods. Groups were compared using the Wilcoxon–Mann–Whitney test, and survival data were analysed using Kaplan–Meier estimates and the log rank test.

The costing data are presented using mean costs with 95 per cent confidence intervals (c.i.). Not all patients had reinterventions; of those who did, reinterventions occurred at different times after the initial placement and were either

Table 1 Comparison of baseline characteristics

	Plastic (*n* = 25)	Metallic (*n* = 25)
Age (years)*	72·3(13·0)	72·9(10·0)
Duration of symptoms (months)*	4·1(2·9)	5·7(3·7)
Length of stricture (cm)*	6·9(2·8)	8·0(3·8)
Distance from incisors (cm)*	30·6(1·0)	30·4(1·0)
Cardia involved	12	9
Histology		
Squamous carcinoma	10	13
Adenocarcinoma	14	12
Other	1	0
Metastatic spread	9	16
Dysphagia score		
4	1	1
3	13	11
2	11	12
1	0	1
Previous treatment		
Laser	1	2
Dilatation	2	4
Radiotherapy	2	4

*Values are mean(s.d.)

Table 2 Finding at reintervention and resultant treatment

	No. of patients*	
	Plastic	Metallic
Finding		
Tumour overgrowth	5	6
Tumour ingrowth	1†	3
Migration	3	2
Tracheo-oesophageal fistula	0	1
Food impaction	5	0
Other	1	1
Treatment		
Endoscopic clearance	5 (7)	0 (0)
Laser	4 (10)	5 (9)
Radiotherapy	1 (1)	0
Metallic stent	3 (3)	6 (8)
Other	2 (5)	6 (6)

Values in parentheses are number of episodes. *Patients may be in more than one category. †Failed plastic prosthesis at initial placement, metallic stent inserted

inpatient or day-case episodes. To account for this, a cumulative cost profile was established for each patient from the time of the initial placement for 1 year. From this, the cumulative mean cost was compared for the two groups at selected timepoints after the initial placement. The mean cost per day survived was calculated by dividing the cumulative cost per patient by the length of survival. Sensitivity analysis based on routine hospital costs was performed.

Results

The two groups were similar, although more patients in the metallic group had metastatic spread (defined as blood-borne metastases) than in the plastic group (*Table 1*). Two patients in the plastic group died after randomization but before placement of a prosthesis.

Technical success and complications within 24 h

Excluding the two patients who died before placement of a prosthesis, there was one placement failure in the plastic group; a metallic stent was subsequently placed. All metallic stents were placed successfully. Pain was the main complication, in 14 patients with a plastic prosthesis and 11 with a metallic stent. Aspiration/reflux was observed in three and four patients respectively. Stent migration, haemorrhage and a perforation occurred in one patient each in the metallic group. The latter complication resolved with conservative management.

Procedural and 30-day mortality

There was no procedure-related death within 24 h of placement in either group. Five patients in the plastic group and six in the metallic group died within 30 days.

Dysphagia scores

Dysphagia scores at follow-up were ranked and compared with baseline as an improvement or deterioration in score ranging from 3 to 0. Dysphagia improved initially in all patients. At 1 month no patient had worsening dysphagia; 13 patients in the plastic group and 14 in the metallic group showed improvement. At 2 months five patients in the plastic group and 12 in the metallic group still showed an improvement compared with baseline. Although there was a trend towards metallic stents being more effective than plastic endoprostheses in improving dysphagia at 2 months, the numbers were small and failed to achieve statistical significance.

Reinterventions

Eight patients (with 26 episodes) in the plastic group and ten patients (with 23 episodes) in the metallic group required further intervention. Recurrent dysphagia (plastic group: seven patients with 21 episodes; metallic group: eight patients with 15 episodes) or bleeding (plastic group: two patients with four episodes; metallic group: five patients with ten episodes) were the commonest symptoms. The

Table 3 Mean cost per patient of initial placement of a plastic prosthesis or metallic stent

	Cost (£)*		
	Plastic (*n* = 25)	Metallic (*n* = 25)	Cost difference (£)†
Prosthesis	128·33(32·60)	762·13(27·60)	633·80 (547·87, 719·74)
Other equipment	70·99(7·91)	113·41(27·94)	42·42 (– 17·09, 101·92)
Drugs	1·08(0·21)	5·26(0·14)	4·18 (3·66, 4·68)
Staff	19·54(2·07)	20·38(2·17)	0·84 (– 5·21, 6·87)
Time in endoscopy, radiology or theatre	49·26(3·22)	46·83(0·97)	– 2·43 (– 9·32, 4·45)
Screening	26·54(4·11)	34·95(1·59)	8·41 (– 0·57, 17·40)
Total cost	295·74(41·53)	982·96(7·04)	687·20 (600·51, 773·90)

Values are *mean(s.e.) or †mean (95 per cent confidence interval) for metallic *versus* plastic

findings at reintervention and resultant treatment are shown in *Table 2*.

Food impaction was restricted to the plastic group and treated endoscopically. Ingrowth occurred only in patients with a metallic stent. Tumour overgrowth was seen in five patients in the plastic group and six in the metallic group. This was treated by laser in the plastic group and by either laser or placement of a second metallic stent in the metallic group. Of patients who experienced bleeding, four in the metallic group required a blood transfusion.

Quality of life

Analysis was restricted to the first 4 months, owing to the small number of patients who survived beyond this point. No statistically significant difference was observed between the groups for any component. However, there was a trend in favour of metallic stents in 21 of the 26 components. This was most apparent for components associated with emotional function ($P = 0{\cdot}16$), insomnia ($P = 0{\cdot}19$) and the symptom of a dry mouth ($P = 0{\cdot}12$).

Survival and medium-term results

Median survival in the plastic group was 62 (range 0–431) days and 107 (range 4–462) days in the metallic group. The estimated relative hazard ratio was 0·66 (95 per cent c.i. 0·37 to 1·16). This trend in favour of metallic stents occurred even though 16 patients in the metallic group had metastatic spread at presentation compared with only nine in the plastic group. Five patients in the metallic group received adjuvant chemotherapy. Analysis of the survival times in these five patients did not show any increase in survival that would explain the differences shown in the whole group (data not shown). Overall, six plastic endoprostheses migrated, two more than 5 cm and four less than 5 cm. Two metallic stents migrated, both in excess of 5 cm; one migrated completely into the stomach.

Cost estimations

The mean cost of the initial placement was £687·21 more in the metallic group than the plastic group, owing to the cost of the metallic stent (mean cost difference £633·80) (*Table 3*). There was no significant difference in the mean cost of placement-related reinterventions between the two groups (plastic: £285·65; metallic: £450·56; mean cost difference £164·91 (95 per cent c.i. –£35·17 to £364·99)). The cumulative mean costs for the two groups were compared at selected timepoints after the initial placement (*Table 4*). Mean costs were higher for the metallic group than for the plastic group at all timepoints, but this was only statistically significant for the first 4 weeks following placement.

The crude mean(s.e.) cost per day survived was £60·40(16·32) for the plastic group and £85·58(22·83) for the metallic group. The mean cost difference was not significant: £25·18 (95 per cent c.i. –£31·25 to £81·60). Inclusion of the cost of hospital stays unrelated to the placement of prostheses increased the mean cost per day survived to £83·11(18·75) in the plastic group and £110·11(22·34) in the metallic group. However, the cost difference was still not significant: £27·00 (95 per cent c.i. –£32·24 to £86·25). A sensitivity analysis using average national costs had little effect on the differential cost of the two procedures (data not shown).

Discussion

Few randomized trials have compared palliative treatments for oesophageal cancer. Such studies are essential to assess the clinical and cost effectiveness of metallic stents, which

Table 4 Cumulative mean costs and cost difference at selected timepoints after initial placement

	Cost (£)*		
Time after placement	Plastic (*n* = 25)	Metallic (*n* = 25)	Cost difference (£)†
Day 0	295·74(41·53)	982·95(7·04)	687·21 (600·51, 773·90)
1 week	1423·32(145·16)	2222·80(119·03)	799·48 (421·67, 1177·30)
2 weeks	1764·07(230·69)	2551·06(204·04)	786·99 (167·51, 1406·46)
3 weeks	1906·09(284·89)	2672·78(241·40)	766·69 (15·38, 1518·01)
4 weeks	1996·56(330·75)	2852·27(292·64)	855·71 (– 32·57, 1743·99)
12 weeks	2355·91(378·43)	2935·47(315·52)	579·56 (– 411·92, 1571·04)
26 weeks	2581·95(468·80)	3740·98(473·70)	1159·03 (– 180·96, 2499·04)
52 weeks	2837·07(520·14)	4224·34(598·51)	1387·28 (– 207·84, 2982·39)

Values are *mean(s.e.) or †mean (95 per cent confidence interval) for metallic *versus* plastic

are significantly more expensive to purchase than plastic endoprostheses[16,17].

In designing this study, a key issue was obtaining robust clinical data on which to base the sample size calculation. At that time, 1995–1996, several case series and comparative studies had been published[18–28], along with one randomized controlled trial with 42 patients[4]. These studies showed that insertion of plastic endoprostheses was associated with higher rates of procedure-related death and complications, in particular perforation of the oesophagus, compared with insertion of metallic stents. However, the reported mortality and perforation rates associated with plastic endoprostheses (up to 16 and 20 per cent respectively) were felt to be much higher than those observed in the present authors' centres. Basing a sample size calculation on these figures would have led to an underestimate in the number of patients required to show a clinically and statistically significant difference. In addition, there was a lack of data examining the quality of life for patients, with only one study reporting quality of life following endoscopic intubation using a non-cancer-specific quality of life measure[29]. On this basis, the study statistician advised that the study be designed as a pilot to obtain data to inform a larger multicentre randomized clinical trial. Statistically significant results were therefore not expected. None the less, some interesting results have been observed and this is still the second largest trial to evaluate the effectiveness of metallic stents in the palliation of oesophageal cancer.

There was no evidence of a difference in procedure-related complications or mortality rate between the two groups. There are several possible reasons for this: only dilatation to a mean of 15 mm was used in the plastic group and conscious sedation was used in most patients. Three of the published trials found a significant difference in procedural complications, e.g. perforations, and mortality rate in favour of metallic stents[5,7,8]. In each, patients receiving a plastic endoprosthesis were dilated to at least 20 mm, and had a general anaesthetic in the process. In contrast, Roseveare *et al.*[6] dilated the plastic group to 17 mm, using conscious sedation. As in the present study, there was no evidence of a difference in the rate of complications, hospital mortality or 30-day mortality. Thus, it may be that the differences in morbidity and procedural mortality observed in other studies are related to the method of insertion of the plastic endoprosthesis.

The mortality rate at 1 month was high in both groups, with one in five patients dying by day 30, although this is similar to other reported rates[4,7,30]. Review of these patients found no evidence to suggest that death was associated with procedural complications. The mortality rate probably reflects the advanced state of disease in these patients; nine of 25 patients who received a plastic endoprosthesis and 16 of those who had a metallic stent had metastatic spread.

This study also observed a trend towards significance in favour of metallic stents for two important outcome measures: quality of life and survival. Quality of life has been assessed in only one other randomized controlled trial, in which there was no difference in either quality of life or enjoyment of food between the metallic and plastic groups[6]. However, the instruments used were not specific to oesophageal cancer. The present study used the EORTC QLQ-C30, a multidimensional cancer-specific measure of quality of life and included an oesophageal cancer-specific component[10,11], allowing quality of life to be measured over 26 components relating to cancer in general and oesophageal cancer in particular. Although none reached statistical significance, 21 of the 26 components measured trended towards the metal group, five were neutral and none favoured plastic prostheses. Oesophageal cancer is a disease with a poor prognosis, and improvements in quality of life represent an important outcome. A larger trial of approximately 300 patients would, however, be required to demonstrate whether the emerging pattern in favour of metallic stents is genuine.

Median survival was greater in the metallic group (but not significantly), even though there were more patients with metastatic disease in the metallic group. Only one other randomized controlled trial has demonstrated a survival trend in favour of metallic stents over plastic endoprostheses[6], which was attributed to better dysphagia scores and nutritional status with improved maintenance of bodyweight. The reason for the increased survival in the present study is not clear. Weight was not recorded and there was no significant difference in dysphagia scores, although the trend at 2 months was in favour of improved dysphagia in patients with metallic stents. The contribution of food impaction, which was restricted to the plastic group, may be relevant to this finding. In addition, several quality of life components likely to be associated with enjoyment of food also tended to favour metallic stents, including nausea and vomiting, problems with eating, dysphagia, deglutition and dry mouth. Finally, the increased diameter achieved by metallic stents without the risks of dilatation associated with plastic endoprostheses may also be important.

Metallic stents were initially more expensive than plastic endoprostheses. There was no difference, however, in the cost of later reinterventions between the two groups and the cost difference between the groups became non-significant 4 weeks after placement. As patients who received metallic stents survived longer, the mean cost per day survived was the same in both groups.

While the study was successful in its original aim of obtaining data to inform a larger study evaluating the effectiveness of metallic stents, it has been overtaken by events within the health service. A recent audit in Scotland of palliative treatments for oesophageal cancer showed that metallic stents are now the commonest type of palliative treatment, currently used in 40 per cent of patients. This situation of clinical practice overtaking research evidence is not unique[31]. However, rigorous evaluation of the effectiveness of new treatments and procedures requires time. In this case, a relatively small trial recruiting 50 patients took 5 years, from the initial design of the trial to the final reporting. As the requirement for good clinical practice to be based on evidence grows, the real issue of the time required to produce that evidence must be acknowledged.

In conclusion, this study found no significant difference in procedure-related complications or mortality, or in 30-day mortality rates between patients palliated with a plastic endoprosthesis or a self-expanding metallic stent. There was a trend towards significance in favour of metallic stents for quality of life and survival. Although there is no clear explanation for the possible survival benefit of metallic stents, it may be related to the lack of food impaction in the metallic stent group and to a greater enjoyment of food, as suggested by the quality of life scores. Although the cost of initial placement was higher in the metallic stent group, this cost difference did not last beyond 4 weeks. A multicentre randomized trial of approximately 300 patients is still urgently required to determine the clinical and cost effectiveness of the use of metallic stents in the palliation of oesophageal cancer.

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Randomized clinical trial to determine if delay from time of penetrating colonic injury precludes primary repair

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Background: There is often a delay of more than 12 h in transferring patients with penetrating colonic injury from outlying hospitals to a regional referral centre. The aim of this prospective study was to determine whether primary suture of a penetrating colonic injury in the presence of delayed presentation, shock, peritoneal contamination or associated injuries leads to increased morbidity and mortality rates.

Methods: Patients with penetrating colonic injuries were randomized to primary closure or colostomy. Patients were compared with regard to interval from injury to operation, associated injuries, duration of operation, postoperative complications and hospital stay.

Results: Two hundred and forty patients were seen over a 69-month period. The interval from injury to operation ranged from 3 to 56 h, and was similar in the two treatment groups. Postoperative complications were similar in the two groups but there were significant differences in operation time (mean(s.d.) 127·1(45·8) min for primary repair and 142·3(43·0) min for colostomy; $P = 0·009$) and length of hospital stay (mean (range) 9 (6–56) *versus* 26 (13–64) days respectively; $P < 0·001$).

Conclusion: Delay from time of penetrating colonic injury is not a contraindication to primary repair.

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Introduction

Although controversies regarding the management of penetrating colonic injury in civilian practice are largely resolved, there are still questions about whether primary closure of such injuries in the presence of delayed presentation, shock, associated injuries or peritoneal contamination leads to increased morbidity and mortality rates.

Penetrating colonic injury has in the past been treated by initial colostomy and later elective closure. Recently primary colonic repair has emerged as the treatment of choice[1–4]. Primary colonic repair avoids the need for a second operation to close the colostomy, and results in a shorter operation time and overall hospital stay[1,4–6]. Colostomy, on the other hand, may be associated with complications related to the stoma, increased costs and the morbidity of waiting for closure[1,4,7–9,10].

Intraoperative prograde colonic lavage confers no significant advantage in the management of penetrating colonic injury[11]. Exteriorized repair has also been shown to be associated with adverse outcomes[10,12]. In many centres the results of primary suturing are so superior that colostomy has become obsolete in the treatment of these injuries[1,13–20].

The Medical University of Southern Africa (MEDUNSA)/Ga-Rankuwa is a university teaching hospital that serves the surrounding periurban communities in addition to being a referral centre for the Northern Province and parts of the Northwest and Mpumalanga Provinces of South Africa. Some of the outlying referring hospitals are more than 500 km away and a significant proportion of patients with trauma who are referred to MEDUNSA/Ga-Rankuwa Hospital have a preoperative injury period of greater than 12 h. This study was undertaken to determine whether primary repair of penetrating colonic injuries, as an alternative to colostomy, can safely be performed in conditions of delayed presentation, shock, faecal contamination or associated intra-abdominal injury without increasing morbidity and mortality rates.

Patients and methods

This prospective randomized study started in January 1993 and ended in September 1998. Randomization lists were prepared and tested for randomness in batches of 60 patients each. The study was concluded at the end of the fourth batch when the numbers were considered adequate for statistical analysis. During this period, patients who presented to MEDUNSA/Ga-Rankuwa Hospital with penetrating colon injuries were randomized to either primary repair or colostomy. Informed consent was obtained from all patients with signs of peritoneal irritation before they were subjected to laparotomy. All patients were resuscitated and optimized for surgery according to Advanced Trauma Life Support guidelines, with crystalloids and blood transfusion where necessary. Shock was defined as a systolic blood pressure of 80 mmHg or less. The time from injury to operation and time taken to perform the procedure were recorded. All patients received prophylactic antibiotics (cefuroxime or piperacillin) at the time of induction of anaesthesia. The majority (90 per cent) of operations were performed by senior registrars. Permission to conduct the study was obtained from the Human Ethics Committee of MEDUNSA.

The two groups were compared with respect to complications (anastomotic leak, abscess formation, wound sepsis, trajectory path sepsis, enterocutaneous fistula, drain site sepsis), death, associated injuries, shock, duration of operation, preoperative injury period and hospital stay. Patients were monitored for postoperative complication by daily examination to detect pyrexia, abdominal tenderness, distension and ileus, and also by laboratory investigations. Patients who had primary colonic repair had pencil drains placed near the anastomosis. Patients with signs suggestive of intra-abdominal sepsis underwent ultrasonography and, where indicated, abdominal computed tomography with intravenous contrast.

Associated injuries were scored according to the Penetrating Abdominal Trauma Index (PATI) and Injury Severity Score (ISS). Only the most severely injured organ was scored in patients in whom more than one organ was involved.

The data were analysed at the Medical Research Council of South Africa in Pretoria. Fisher's exact test was used. $P \leqslant 0{\cdot}05$ was considered significant. Statistical regression analysis was used to calculate adjusted odds ratios (OR) and 95 per cent confidence intervals for developing sepsis and prolonged hospital stay for each treatment category and overall for each risk factor (type of surgical treatment, gender, shock, preoperative injury period, blood transfusion, mechanism of injury and associated injuries).

Results

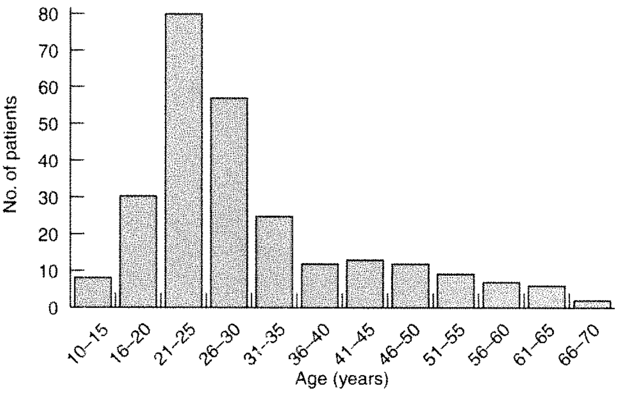

Fig. 1 Age distribution of patients

Two hundred and forty patients with intraperitoneal penetrating colonic injuries were included in the study. Patients with extraperitoneal rectal injuries were excluded as they all underwent colostomy. There were 215 (89·6 per cent) males and 25 (10·4 per cent) females whose age ranged from 12 to 67 (mean 30) years (*Fig. 1*). The majority of injuries (191; 79·6 per cent) were low-energy transfer gunshot wounds and the remaining 49 (20·4 per cent) were stab wounds. Time from injury to operation ranged from 3 to 56 h.

Two groups of patients were defined according to the delay from time of injury to operation; the delay was between 0 and 12 h in group 1 and more than 12 h in group 2. Postoperative complications (septic, pulmonary, wound dehiscence, anastomotic leak, enterocutaneous fistula), death, gender, age, mechanism of injury, shock, blood transfusion, site of colonic injury, mean injury score (PATI + ISS), operation time, hospital stay and type of surgical treatment in the two groups are shown in *Table 1*.

Details of the associated injuries are shown in *Table 2*. The PATI and ISS score was calculated for each preoperative injury group. There were no significant differences between preoperative injury and treatment groups (*Table 1*). Two patients (0·8 per cent) who had a colostomy died from multiple organ failure at day 12 and day 16 after laparotomy. Both had severe associated injuries and died from causes unrelated to the procedure performed.

Thirty patients (12·5 per cent) were clinically shocked at the time of presentation. There was no significant difference in the incidence of shock between the preoperative groups (*Table 1*) and between colostomy and primary repair groups (*Table 3*). A total of 92 patients (38·3 per cent) required blood transfusion median 3 (range 2–6) units. These were evenly distributed between the primary repair and colostomy groups (*Table 3*).

Sepsis included wound, drain site and trajectory path sepsis. One patient with a colostomy had an abscess in the stab wound. Septic complications occurred in 53 patients

Table 1 Distribution of patients related to time of presentation and treatment type

	Group 1 (0–12 h)		Group 2 (> 12 h)		
	Primary repair	Colostomy	Primary repair	Colostomy	Total
Gender					
Male	65 (27·1)	55 (22·9)	43 (17·9)	52 (21·7)	215 (89·6)
Female	8 (3·3)	9 (3·8)	4 (1·7)	4 (1·7)	25 (10·4)
Age (years)*	28·7(11·2)	28·4(11·4)	30·3(10·3)	33·5(14·0)	30·1(11·7)
Mechanism of injury					
Stab	16 (6·7)	9 (3·8)	12 (5·0)	12 (5·0)	49 (20·4)
Gun	57 (23·8)	55 (22·9)	35 (14·6)	44 (18·3)	191 (79·6)
Shock					
No	65 (27·1)	53 (22·1)	42 (17·5)	50 (20·8)	210 (87·5)
Yes	8 (3·3)	11 (4·6)	5 (2·1)	6 (2·5)	30 (12·5)
Blood transfusion					
No	49 (20·4)	33 (13·8)	25 (10·4)	41 (17·1)	148 (61·7)
Yes	24 (10·0)	31 (12·9)	22 (9·2)	15 (6·3)	92 (38·3)
Site of colon injury					
Ascending	20 (8·3)	15 (6·3)	14 (5·8)	18 (7·5)	67 (27·9)
Transverse	32 (13·3)	28 (11·7)	11 (4·6)	17 (7·1)	88 (36·7)
Descending	9 (3·8)	12 (5·0)	12 (5·0)	9 (3·8)	42 (17·5)
Sigmoid	11 (4·6)	9 (3·8)	10 (4·2)	13 (5·4)	43 (17·9)
Associated injuries*†	4·7(3·9)	5·3(3·7)	4·4(3·8)	5·2(3·9)	4·9(3·8)
Operation time (min)*	124·3(48·7)	145·6(48·0)	131·6(41·0)	138·4(36·6)	134·7(45·0)
Hospital stay (days)					
< 15	53 (22·1)	21 (8·8)	36 (15·0)	16 (6·7)	126 (52·5)
> 15	20 (8·3)	43 (17·9)	11 (4·6)	40 (16·7)	114 (47·5)
Sepsis					
No	51 (21·3)	49 (20·4)	38 (15·8)	49 (20·4)	187 (77·9)
Yes	22 (9·2)	15 (6·3)	9 (3·8)	7 (2·9)	53 (22·1)
Pulmonary complications					
No	68 (28·3)	62 (25·8)	46 (19·2)	55 (22·9)	231 (96·2)
Yes	5 (2·1)	2 (0·8)	1 (0·4)	1 (0·4)	9 (3·8)
Wound dehiscence					
No	71 (29·6)	64 (26·7)	47 (19·6)	54 (22·5)	236 (98·3)
Yes	2 (0·8)	0 (0·0)	0 (0·0)	2 (0·8)	4 (1·7)
Anastomotic leak					
No	70 (29·2)	64 (26·7)	47 (19·6)	56 (23·3)	237 (98·7)
Yes	3 (1·3)	0 (0·0)	0 (0·0)	0 (0·0)	3 (1·3)
Death					
No	73 (30·4)	64 (26·7)	47 (19·6)	54 (22·5)	238 (99·2)
Yes	0 (0·0)	2 (0·8)	0 (0·0)	0 (0·0)	2 (0·8)
Enterocutaneous fistula					
No	72 (30·0)	64 (26·7)	46 (19·2)	54 (22·5)	236 (98·3)
Yes	1 (0·4)	0 (0·0)	1 (0·4)	2 (0·8)	4 (1·7)

Unless otherwise indicated values in parentheses are percentages; *values are mean(s.d.). †Penetrating Abdominal Trauma Index plus Injury Severity Score

(22·1 per cent); there was no significant difference in their distribution between patients who had primary closure (31 patients; 25·8 per cent) or colostomy (22 patients; 18·3 per cent) (*Table 1*). The majority of these septic episodes were due to wound sepsis (primary repair 25 (20·8 per cent) and colostomy 17 (14·2 per cent)).

Three patients required colonic resection; two had right hemicolectomy with ileotransverse anastomosis and the third underwent resection of the transverse colon with colostomy. All recovered without complication. Three patients (1·3 per cent) who had primary closure had an anastomotic leak; two leaks presented clinically and one was discovered at repair of a dehisced abdominal incision wound.

All patients who had an anastomotic leak or postoperative wound dehiscence and two patients who had an enterocutaneous fistula required reoperation. The fistulas required reintervention after conservative methods had failed. Eleven of the 42 septic wounds required secondary suturing after sepsis control. The decision and timing of reintervention depended on the surgeon in charge of the patient.

Table 2 Details of associated injuries

Associated injury	Primary repair	Colostomy	Total
Small bowel	42	44	86 (35·8)
Stomach	22	15	37 (15·4)
Duodenum	3	3	6 (2·5)
Appendix	3	0	3 (1·3)
Liver	14	9	23 (9·6)
Diaphragm	6	10	16 (6·7)
Spleen	6	3	9 (3·8)
Gallbladder	6	3	9 (3·8)
Kidney	11	6	17 (7·1)
Ureter	2	3	5 (2·1)
Bladder	0	3	3 (1·3)
Uterus	3	0	3 (1·3)
Extra-abdominal*	29	23	52 (21·7)

Values in parentheses are percentages. *Fractures, chest, neurovascular and external genitalia

Table 3 Comparison between primary repair and colostomy groups regarding complications and hospital stay

	Primary repair	Colostomy	*P*
Sepsis	31 (25·8)	22 (18·3)	0·21
Wound	25 (20·8)	17 (14·2)	0·23
Drain site	4 (3·3)	3 (2·5)	1·00
Trajectory path	2 (1·7)	2 (1·7)	1·00
Pulmonary complications*	6 (5·0)	3 (2·5)	0·50
Wound dehiscence	2 (1·7)	2 (1·7)	1·00
Enterocutaneous fistula	2 (1·7)	2 (1·7)	1·00
Death	0 (0·0)	2 (1·7)	0·50
Anastomotic leak	3 (2·5)	0 (0·0)	0·25
Shock	13 (10·8)	17 (14·2)	1·00
Blood transfusion	46 (38·3)	46 (38·3)	1·00
Hospital stay > 15 days	31 (25·8)	83 (69·2)	< 0·001

Values in parentheses are percentages. *Acquired respiratory distress syndrome

There was a significant difference in overall duration of hospital stay between the primary repair and colostomy groups (mean (range) 9 (6–56) *versus* 26 (13–64) days respectively; $P < 0{\cdot}001$). These data included admission for the initial operation and that for closure of the colostomy. Colostomy procedures took longer than primary repair procedures (mean(s.d.) (range) 127·1(45·8) (60–225) min for primary repair and 142·3(43·0) (68–270) min for colostomy; $P = 0{\cdot}009$).

Table 4 shows adjusted ORs for developing sepsis and prolonged hospital stay for each treatment category and overall for each risk factor indicated. For sepsis, there was a marked difference in adjusted OR between primary closure and colostomy for the risk factors mechanism of injury and associated injuries. There was marked difference in duration of hospital stay for the risk factors shock, blood transfusion, mechanism of injury, associated injuries and type of surgical treatment.

Discussion

Traditionally, delayed treatment of colonic injuries has been considered to predispose to postoperative morbidity. Some investigators have suggested a cut-off time of 12 h after injury beyond which primary repair cannot be performed safely[6]. Others have also recommended colostomy for patients who are shocked at presentation or who have multiple associated injuries[3,8,21].

In this study, the postoperative morbidity was similar after primary repair and colostomy, and in patients with a preoperative injury period of less than 12 h and those with a longer interval between injury and operation. There was no difference in postoperative complication rate after primary repair and colostomy in patients who were shocked, required blood transfusion and had associated injuries. Patients with a colostomy had a longer hospital stay because of the need to learn stoma care.

A few drain sites became infected and two of three anastomotic leaks were demonstrated by discharge through these drains. It is not absolutely necessary to leave a drain near the repair/anastomotic site[22]. Sepsis in the bullet trajectory path was in the exit rather than the entrance path and more so in those who presented late. It is recommended that the exit path be debrided thoroughly and washed clean[23]. All incision wounds were closed primarily. To reduce the risk of wound infection, some investigators have suggested that the skin should be left open rather than closed primarily in patients with gross contamination and a high colon injury scale[24]. In the current study it was difficult to quantify contamination and as such it was not possible to include this variable in analysis of the data. Intra-abdominal soiling should not be a contraindication to primary repair with effective antibiotic treatment[25].

The majority of the patients who had primary closure had a single follow-up visit to the hospital after discharge, whereas those with a colostomy had several follow-up visits between the initial laparotomy and closure of the colostomy, and after colostomy closure.

There was a total of 15 complications related to the management of colonic perforation (6·3 per cent). There was no significant difference in the incidence between colostomy and primary repair groups. Two patients required resection with primary anastomosis. Both presented late (after 12 h) and recovered without any complications. In the American Association for the Surgery of Trauma prospective multicentre study of penetrating colonic injuries requiring resection, Demetriades *et al.*[26] found that the method of management

Table 4 Risk of sepsis and prolonged hospital stay

		Sepsis			Hospital stay		
			Adjusted OR†			Adjusted OR†	
Risk factor	Total no. of patients*	No. of patients*	By surgery group	Overall	No. with prolonged stay*‡	By surgery group>	Overall
Colon treatment				0·6 (0·3, 1·1)			6·4 (3·6, 11·5)
Primary repair	120 (50·0)	31 (12·9)			31 (12·9)		
Colostomy	120 (50·0)	22 (9·2)			83 (34·6)		
Shock				1·3 (0·5, 3·4)			2·9 (0·9, 5·4)
Primary repair			1·6 (0·4, 5·8)			1·5 (0·4, 5·4)	
No	107 (44·6)	26 (10·8)			26 (10·8)		
Yes	13 (5·4)	5 (2·1)			5 (2·1)		
Colostomy			1·3 (0·2, 6·9)			10·0 (1·1, 89·8)	
No	103 (42·9)	18 (7·5)			69 (28·8)		
Yes	17 (7·1)	4 (1·7)			14 (5·8)		
Preoperative injury period				0·4 (0·3, 1·0)			1·1 (0·5, 4·1)
Primary repair			0·5 (0·2, 1·3)			0·8 (0·3, 1·9)	
< 12 h	73 (30·4)	22 (9·2)			20 (8·3)		
⩾ 12 h	47 (19·6)	9 (3·8)			11 (4·6)		
Colostomy			0·2 (0·0, 0·9)			1·4 (0·5, 3·8)	
< 12 h	64 (26·7)	15 (6·3)			43 (17·9)		
⩾ 12 h	56 (23·3)	7 (2·9)			40 (16·7)		
Blood transfusion				1·7 (0·8, 3·1)			1·1 (0·6, 2·1)
Primary repair			1·9 (0·7, 4·6)			1·8 (0·7, 4·4)	
No	74 (30·8)	15 (6·3)			15 (6·3)		
Yes	46 (19·2)	16 (6·7)			16 (6·7)		
Colostomy			1·4 (0·4, 5·3)			0·6 (0·2, 1·6)	
No	74 (30·8)	12 (5·0)			51 (21·3)		
Yes	46 (19·2)	10 (4·2)			32 (13·3)		
Mechanism of injury				4·3 (1·1, 7·9)			2·3 (1·0, 4·6)
Primary repair			15·9 (1·8, 141·9)			3·8 (1·0, 14·8)	
Stab	28 (11·7)	1 (0·4)			3 (1·3)		
Gun	92 (38·3)	30 (12·5)			28 (11·7)		
Colostomy			1·3 (0·2, 7·1)			1·2 (0·3, 4·7)	
Stab	21 (8·8)	4 (1·7)			13 (5·4)		
Gun	99 (41·3)	18 (7·5)			70 (29·2)		
Associated injuries§				1·0 (0·5, 3·1)			1·4 (0·6, 3·4)
Primary repair			3·4 (0·5, 23·6)			2·3 (0·4, 12·4)	
< 10	112 (46·7)	28 (11·7)			28 (11·7)		
⩾ 10	8 (3·3)	3 (1·3)			3 (1·3)		
Colostomy			–			0·9 (0·1, 5·5)	
< 10	114 (47·5)	15 (6·3)			88 (36·7)		
> 10	6 (2·5)	0 (0·0)			4 (1·7)		

Values in parentheses are *percentages or †95 per cent confidence intervals. ‡More than 15 days. OR, odds ratio. §Penetrating Abdominal Trauma Index plus Injury Severity Score

of the colonic perforation (primary repair *versus* colostomy) did not influence the incidence of complications related to the colon. The authors concluded that such injuries should be managed by primary repair[26].

This study has demonstrated that primary repair of civilian penetrating injury of the colon is safe irrespective of the delay in presentation, shock, blood transfusion, intra-abdominal soiling and associated injuries. The postoperative morbidity and mortality rates are comparable to those of colostomy, but primary repair has the added advantages of a shorter operation time, shorter hospital stay, reduced costs and better quality of life. Primary repair should be the procedure of choice for penetrating colon injuries in such patients.

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Original article

Use of electrolysis as an adjunct to liver resection

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Background: Patients with hepatic metastases are potentially curable if all the diseased tissue can be resected. Unfortunately, only 10–20 per cent of patients are suitable for curative resection. Electrolysis is a novel non-thermal method of tissue ablation. When used in conjunction with surgery it may increase the number of resectable liver tumours with curative treatment.
Methods: All patients had been deemed inoperable using currently accepted criteria. Nine patients with hepatic deposits from colorectal carcinoma underwent combined surgical resection and electrolytic ablation of metastases.
Results: The treatment was associated with minimal morbidity. Within the electrolytically treated area seven patients had no radiological evidence of recurrence at a median follow-up of 9 (range 6–43) months; local recurrence was detected in two patients. Six of the nine patients had metastases elsewhere in the liver with four having extrahepatic metastases. Three patients remain tumour free. Three patients died. The median survival was 17 (range 9–24) months from the time of treatment.
Discussion: Electrolysis with resection may confer a disease-free and overall survival benefit. The small size of this initial study precludes statistical analysis, but preliminary results are encouraging.

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Introduction

Colorectal carcinoma is the third most common malignancy and the most common source of liver metastases, with the liver being the earliest and often the only site of metastatic disease[1]. These patients are potentially curable if all diseased tissue can be removed[2,3], but have a poor prognosis (measured in months) if this is not feasible[4]. The size, number or position of the metastatic lesions allows only 10–20 per cent of patients to be eligible for a formal resection[5].

Several ablative techniques have been investigated as alternatives to surgical resection for the treatment of colorectal metastases[4,6]. Electrolysis is a novel non-thermal method of tissue ablation[7]. A direct current is passed through a conductive medium between a pair of electrodes (cathode and anode). The electrolytic 'dose' is measured in coulombs (amperes × seconds). Tissue electrolysis produces sodium hydroxide and hydrogen at the cathode, and hydrochloric acid and chlorine gas at the anode[8,9]. The significant pH changes produced by electrolysis are cytotoxic and cause localized parenchymal necrosis with a negligible thermal effect[7–9].

Over the past few years electrolysis of liver tumours has been investigated extensively at this institution[7,10–13]. Not only has the process been shown to ablate liver tumours completely in experimental models[12,14], but it is also safe in terms of both its inherent tissue destruction and the inability to thrombose or damage large blood vessels in close proximity to the electrolytic lesion[13]. A pilot study was performed in five patients before tissue resection to determine whether this method could be used safely. No adverse effects resulted from the treatment (D. Berry, S. Wemyss-Holden, G. J. Madden, A. Dennison, unpublished results). Nine patients were then subjected to a formal liver resection, with electrolytic ablation of metastases that would have deemed the cancer irresectable using currently accepted criteria.

Patients and methods

The records of nine patients with a histologically confirmed diagnosis of secondary liver metastases from colorectal carcinoma between the dates of April 1997 to May 2000 were reviewed. The median age was 57 (range 44–66) years.

The Editors have satisfied themselves that all authors have contributed significantly to this publication

Conventional surgical treatment was not possible because of the multiplicity of tumours, proximity to major vessels or distribution of metastases, or a combination of these factors. Patients were excluded from the study if they were ineligible for laparotomy, or had extrahepatic disease or multifocal liver metastases such that resection and ablation would compromise liver function. All patients included in the study had previously undergone resection of a primary colorectal tumour. Four patients had previously undergone hepatic resection and presented with irresectable hepatic recurrence over a period of 37 months (August 1997 to September 2000). One patient had undergone previous hepatic resection and was receiving electrolysis alone for a single irresectable metastatic deposit.

The patients underwent formal resection of hepatic metastases, followed by electrolytic ablation of metastases that could not be resected surgically. Ethics committee approval was obtained before any of the patients was treated. Before electrolysis, all patients were fully informed about the experimental nature of the study.

Two 6-Fr electrolysis catheters (Cordis Webster, Johnson and Johnson Medical, North Ryde, New South Wales, Australia) were inserted into the tumour, 9–24 mm apart, depending on the size of the tumour. Electrode positioning was confirmed ultrasonographically. The catheters were attached to a direct current generator (Medizintechnic, Soring, Quickborn, Germany), and a predetermined 'dose' was delivered at a constant current, calculated for the desired volume of necrosis. Tumour dimensions and position were determined using previous computed tomography (CT) scans and confirmed by intraoperative ultrasonography. The volume of each lesion was calculated using the formula $V = 4/3\pi(r_1 \times r_2 \times r_3)$. A 5-mm margin of normal parenchyma surrounding the tumour was also ablated. Previous experimental studies had shown a linear dose–response of 3·5 cm^3 per 100 coulombs (C)[10]. Tumour radius ranged from 5 to 30 mm. The 'dose' varied from 200 to 1000 C according to the calculated volume. The length of time taken for electrolysis ranged from 42 to 210 min. Platinum was chosen as the electrode material because of its inert nature, and theoretical antineoplastic properties[15].

White cell count and liver function tests were carried out before and after treatment. Carcinoembryonic antigen (CEA) levels were monitored after operation to assist in determining any tumour recurrence.

CT scans were reviewed retrospectively by a radiologist, who was blinded to treatment details for each patient. The radiologist interpreted and compared CT findings before electrolysis with those obtained at 1 week, 3, 6 and 9 months, and annually after electrolysis to determine the effectiveness of treatment. The electrolytic lesion was evaluated for size, shape and location following electrolysis as well as for evidence of recurrence. Successful treatment was associated with no tumour recurrence within the electrolytically treated lesion, while failure of electrolytic treatment was associated with recurrence within the treated area. Recurrence elsewhere was considered to be due to the natural course of the metastatic disease and not a failure of treatment.

Results

Of the nine patients treated, six are still alive. The procedures were performed with a median hospital stay of 9 days. Median follow-up was 9 (range 6–43) months. In two patients who had electrolysis of lesions lying adjacent to the inferior vena cava, there was no damage to the vascular structure.

Seven of the nine patients with hepatic metastases from colorectal carcinoma showed no evidence of radiological recurrence within the electrolytically treated lesion in the follow-up period. In each of these patients, electrolytic treatment was deemed successful. Recurrence within the electrolytically treated area occurred in two patients.

Six of the nine patients experienced hepatic recurrence of tumour outside the electrolytically treated area, which was not related to the previously treated area. There was no evidence of recurrence around the position of the electrodes due to seeding in any patient. Four patients had extrahepatic disease that was histologically confirmed to resemble adenocarcinoma, consistent with metastasis from the primary colorectal carcinoma.

CEA levels were increased after operation in four of the seven patients who had tumour recurrence. One patient, who experienced shrinkage of the treated tumour and no recurrence, had reduced CEA levels after operation. Liver enzyme levels and white cell counts were raised in the early postoperative period, but returned to normal within 3 months of treatment. No major complications occurred either during or after treatment.

All patients received chemotherapy before or after treatment.

Discussion

In recent years there has been an increasing interest in the use of ablative techniques for treating previously irresectable disease[4,16–19]. These techniques have the advantage of preserving more liver parenchyma, and are associated with fewer complications than resection[4]. The use of ablation as an adjunct to surgical resection is not yet established.

Electrolytic ablation of liver tumours has previously been described in China[20,21], with promising results. It is a non-thermal technique, and therefore not limited by the 'heat sink' effect of nearby vessels[7]. It is also safe in terms of its inherent tissue destruction and inability to thrombose or breach large blood vessels in close proximity to the site of electrolysis[13]. Electrolysis is also predictable, with the volume of the lesion created being proportional to the electrolytic 'dose' (in coulombs) administered[10]. Currently, a limitation of the technique is that it is time consuming, with an increase in tumour size necessitating an exponential increase in treatment time. At present it is limited to lesions of less than 5 cm in diameter and requires a laparotomy. However, further development of appropriate electrode delivery systems may result in the treatment being administered percutaneously.

CT has been used in this study for follow-up evaluation as it has been proven to be sensitive in characterizing hepatic lesions[22]. A small margin of normal tissue at the periphery of the tumour is ablated to prevent residual viable tumour cells persisting at the margin and leading to recurrence. An initial increase in size of created lesions in the first week after operation is expected on the CT scan as this represents ablation of a margin of normal tissue (*Figs 1* and *2*). A decline in lesion size after the first week reflects successful treatment, while an increase in size suggests failure[23]. Conservative electrolytic treatment in the early stages of the trial may have resulted in undertreatment of tumours, allowing residual viable tumour cells to persist within the lesions, causing recurrence. With greater experience more accurate 'doses' of current were delivered, based on tumour geometry.

Necrosis in the electrolytically treated area appears hypodense on CT. Fibrosis within the treated area of some patients is evidence of shrinkage of the electrolytically treated area from a fluid-filled necrotic zone to a smaller fibrosed area (*Fig. 3*). Areas of fibrosis appear enhanced on contrast CT. Magnetic resonance imaging may be useful in evaluating these patients and a study to investigate this has been initiated.

The increase in liver enzyme levels after electrolysis was transient and short-lived. In most patients enzyme levels had returned to normal within 3 months of treatment.

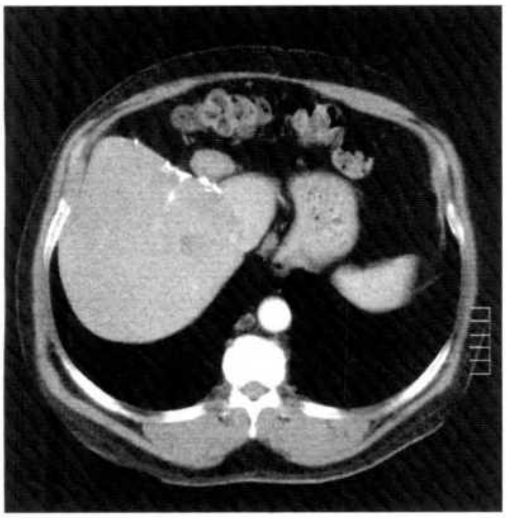

Fig. 1 Computed tomogram of a lesion before treatment

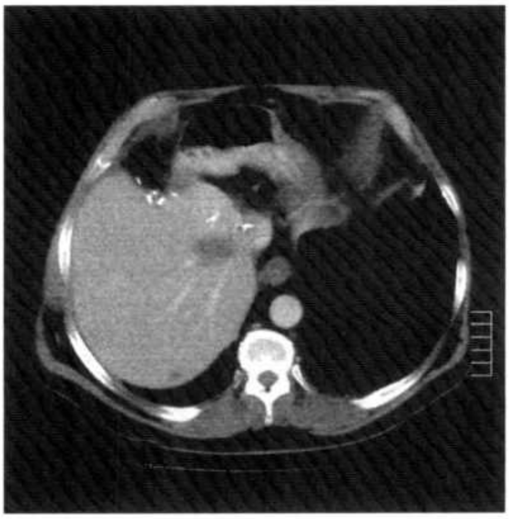

Fig. 2 Computed tomogram of the electrolytically treated lesion in the same patient 1 week after operation, showing ablation of a margin of surrounding normal tissue

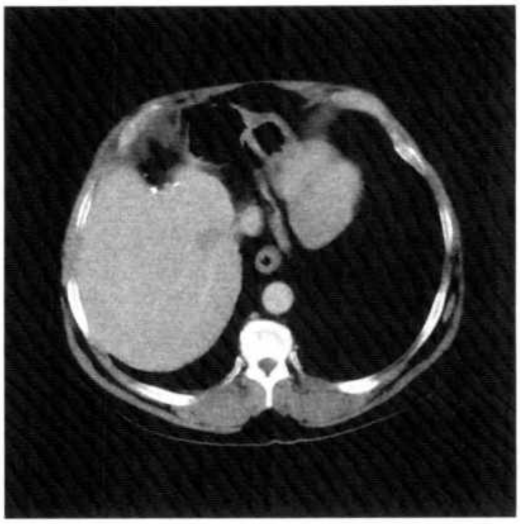

Fig. 3 Computed tomogram of the electrolytically treated lesion in the same patient 3 months after electrolysis, showing fibrosis and shrinkage of the treated area

In patients with colorectal hepatic metastases CEA is a serum tumour marker that can be useful in detecting tumour recurrence. CEA is not associated specifically with colorectal carcinoma but may be raised in up to 70 per cent of patients affected[22]. Four patients had a significantly increased CEA level after electrolysis, consistent with the tumour recurrence noted. One patient in whom treatment was effective had a reduced CEA level following electrolysis.

There was no detectable additional morbidity associated with the electrolytic treatment when used as an adjunct to surgical resection. The lack of major complications associated with electrolysis suggests that it is safe as a method of local ablation.

The median survival from the time of the procedure was 17 (range 9–24) months. This result should be interpreted in the context of the natural history of untreated colorectal liver metastases, for which untreated median survival is of the order of 3–6 months[6,24,25]. The increased survival time in this group of patients receiving electrolysis with resection may have an overall survival benefit. The small size of this initial study precludes statistical analysis of the results. The use of electrolysis in the ablation of liver tumours requires further long-term studies and evaluation; however, preliminary results are encouraging.

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Original article

Experimental study of large-volume microwave ablation in the liver

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Background: Microwave coagulation therapy is useful in the destruction of small, irresectable liver tumours of primary and secondary origin. Unfortunately, the small lesion size produced by currently available equipment makes it difficult and time consuming completely to ablate lesions larger than 3 cm in diameter. A microwave system capable of producing large-volume ablations in very short periods of time has been developed. Using a large-animal model the ability of the equipment to produce large-volume lesions in a safe, predictable and dose-dependent manner was tested.

Methods: Fourteen large white pigs were anaesthetized and underwent multiple microwave treatments. The animals were killed at different timepoints to investigate lesion size and evolution.

Results: The microwave system was able to generate large-volume ablations of up to 6·5 cm in diameter in a controlled and dose-dependent manner.

Conclusion: This novel microwave system allows the ablation of large volumes of liver tissue in a short period of time. The ability to produce lesions reproducibly and safely highlights the potential of this system in the future treatment of irresectable liver tumours.

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Introduction

Microwave coagulation therapy is one of a number of ablative therapies being developed to treat patients with irresectable liver cancers[1]. Destruction of neoplastic cells is induced by local thermal energy generated by a controlled microwave field. Previous investigations have demonstrated the ability of this treatment to cause tumour cell death in both animal models and humans[2–4]. Currently available microwave equipment is able to create an ablated lesion of approximately 2 cm in diameter[5]. Hepatic malignancies 2 cm or greater in diameter require multiple, overlapping insertions of the microwave applicator (probe) to achieve complete tumour ablation. All ablative treatments have limited ablation volumes, and higher rates of tumour recurrence are seen following treatment of larger tumours[6–8].

The University of Bath Medical Device Technology Group (UBMDTG) in association with Leicester Royal Infirmary has produced a new generation of microwave ablation technology. The objectives of this experiment were threefold: first, to test the ability of the microwave equipment to produce areas of ablation in normal porcine liver in a reproducible manner; second, to investigate lesion evolution and produce a dose–response curve; and, finally, to investigate the tolerance and safety of such a procedure in a porcine model. Porcine livers were chosen because they are similar to human liver in terms of both size and physiology[9].

Materials and methods

All animal experimentation was conducted in accordance with the Operation of Animals (Scientific Procedures) Act 1986 after authorization from the University of Leicester Ethical Committee and the Home Office. Fourteen large white, female pigs (body-weight 45–50 kg) were anaesthetized and underwent insertion of a tunnelled central venous catheter, allowing easy administration of fluids and analgesics.

The Editors have satisfied themselves that all authors have contributed significantly to this publication

Each animal underwent a laparotomy to allow good exposure of the liver. The UBMDTG microwave applicator was then inserted into the hepatic parenchyma and a 3-min treatment was applied. To reproduce the conditions in which it is believed the microwave will be used and to assist in the production of a dose–response curve, pigs underwent multiple treatments which in some cases included resection of a liver lobe. The range of powers selected included 36, 50, 100, 150 and 200 W, with each pig receiving a combination of treatments. Adjacent liver lobes, viscera and other organs were protected by placement of moist surgical packs. A microwave detector was used in all operations to ensure that personnel present did not receive microwave dosages in excess of European Economic Community regulations.

The microwave system used included a generator (ETM 200 SC Travelling Wave Tube Amplifier; ETM Electromagnetic, Newark, California, USA) driven by a Hewlett Packard 8350B Sweep Oscillator (Agilent Technologies UK, Wokingham, UK) that produced microwaves at a frequency of 2·45 GHz with variable power available from 0 to 250 W. Microwaves were transmitted along a cable to the hand-held applicator.

Following microwave ablation treatment, the abdominal wall was closed and the skin sutured. Local anaesthetic was injected into the wound and long-acting opiates were administered intravenously for at least 3 days. Blood samples were taken from the central venous catheter for the first week.

Lesion evolution was examined over time; animals were killed at increasing intervals from 0 h to 60 days. Lesion diameters were recorded using callipers; blanched regions were considered to represent the margins of ablation. Liver containing the lesion was sectioned and placed in 10 per cent formalin solution for subsequent histological examination.

Statistical analysis

A general linear model analysis of variance was used to compare lesion diameters at different timepoints and power levels, followed by Tukey's test to compare lesion diameters at different timepoints corrected for power levels. Linear and quadratic regression analysis was used to predict lesion diameter for any given power level. Ninety per cent prediction bands were used so that, using the prediction band, there would be a 95 per cent probability that the lesion diameter in an individual would exceed a specified diameter for any given power level. A significance level of $P = 0.05$ was used. All calculations were performed using the MINITAB statistical package (Minitab, State College, Pennsylvania, USA).

Results

The UBMDTG microwave equipment was able to produce large-volume ablations in each liver. No bleeding was evident from the applicator cavity and the inner region of the lesion appeared desiccated. The majority of lesions produced were spherical and therefore circular on sectioning. At all timepoints, the liver adjacent to the lesions was macroscopically and microscopically normal.

The characteristics and sizes of the lesions changed over the course of the experiment. Immediately following treatment, a spherical volume of ablation was produced. On sectioning, a central blanched region with a surrounding zone, possibly haemorrhagic in nature, was seen. Within the blanched region, small areas of cavitation were noted, being especially numerous nearer the centre of the lesion. These were interpreted as a consequence of steam or bubble formation. Microscopy revealed damaged hepatocytes within the blanched region, particularly towards the lesion centre. The surrounding darker halo revealed an abundance of erythrocytes although the hepatocytes showed no signs of heat damage. Lesion diameters varied according to the power applied; larger diameters were produced by treatments using higher powers. The mean lesion diameter for each power at each time interval investigated is shown graphically (*Fig. 1*). After 24 h, the periphery of most lesions was well demarcated from the surrounding hepatic tissue. There was no significant difference between lesion diameters at 0 h, 24 h, 7 days or 30 days ($P > 0.05$), which were a mean(s.e.m.) of 4·0(0·2), 3·9(0·2), 4·4(0·2) and 3·5(0·3) cm respectively (corrected for dose level), but there was a significant decrease in diameter to 1·7(0·2) cm at 60 days using Tukey's *post hoc* comparisons ($P < 0.05$). At 24 h the blanched area extended into the area previously occupied by the darker halo (*Fig. 2*), and this was confirmed

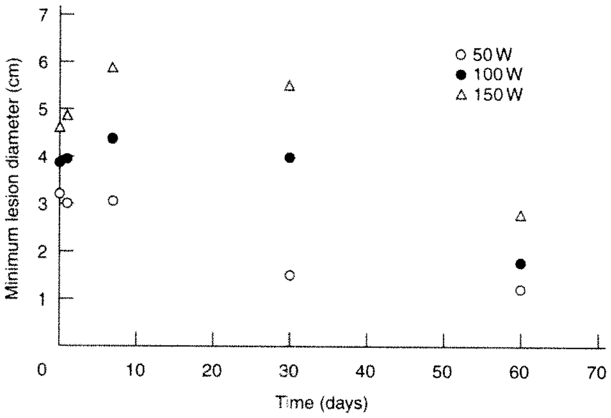

Fig. 1 Mean minimum lesion diameter with time

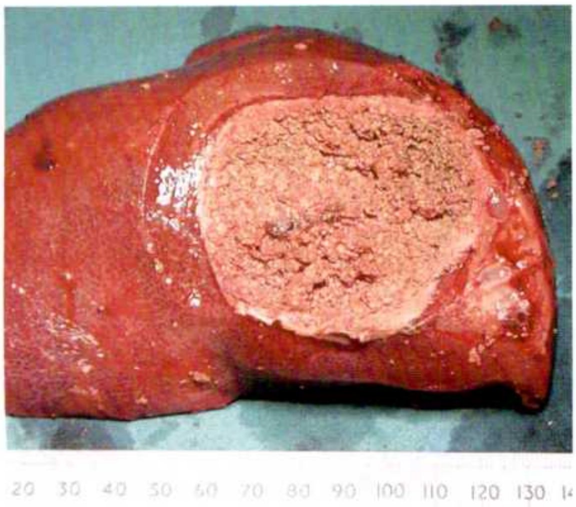

Fig. 2 Lesion created by 150-W microwave sectioned after 24 h. The slightly eccentric extension of the lesion from an otherwise circular shape can be seen in the portion of the liver most distal to the vascular supply

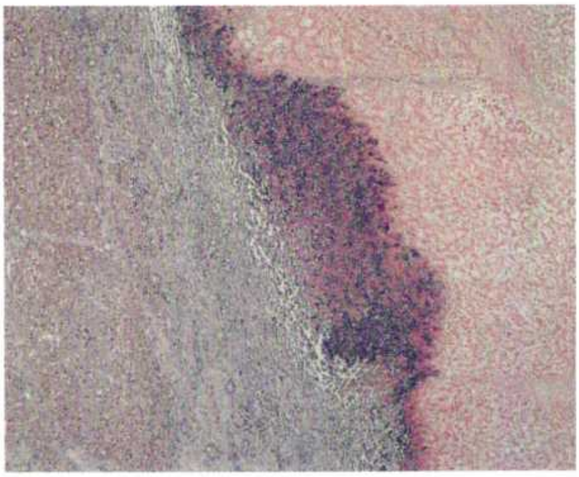

Fig. 3 Section illustrating the sharply demarcated border between ablated, dead liver (right) and normal hepatic parenchyma (left). Between these two areas the fibrous capsule and a rim of darkly staining inflammatory cells are seen. (Haematoxylin and eosin stain, original magnification × 40)

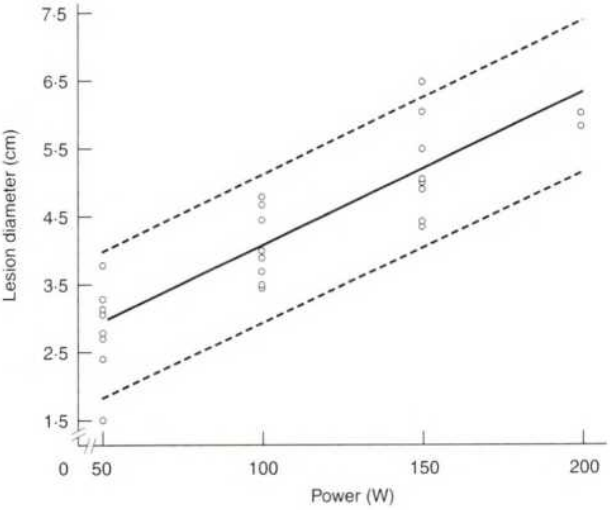

Fig. 4 Regression of lesion minimum diameter on microwave power for all lesions up to and including 30 days after operation. The solid line represents the best estimate of mean diameter for a given power level. The dashed lines show the 90 per cent prediction interval for individual lesions; 95 per cent of lesions are expected to have diameters above the lower dashed line. The point at the 50-W power level with a lesion diameter of 1·5 cm was taken at 30 days and may be the result of shrinkage. (Diameter = 1·8 + 0·022power; $S = 0.61$; $R^2 = 76$ per cent; R^2(adjusted) = 75 per cent)

microscopically. In a number of cases, the extension of the lesion was not entirely uniform; the greatest increases were into the areas of hepatic parenchyma distal to the vascular supply. The material within the lesion confines was not identifiable as normal liver parenchyma and had become a mass of paler, amorphous tissue.

After 1 week, the ablated volumes were well demarcated from the surrounding tissue and the lesion was surrounded by a thin lighter-coloured rim. Microscopy revealed necrotic cells within the lesion, the limit of which was defined by the presence of a fibrous capsule corresponding to the lighter-coloured rim seen macroscopically (*Fig. 3*).

After 30 and 60 days the macroscopic and microscopic appearance of each lesion had not altered from that seen at 1 week. By 60 days, however, the mean lesion diameter had decreased significantly to less than half the diameter seen at preceding intervals ($P < 0.05$). One 50-W lesion had decreased in size dramatically, leaving only an irregular scar. Because there were no significant differences in lesion diameters up to 30 days, all data (excluding diameters at 60 days) were used for the dose–response analysis. Regression analysis showed a highly significant ($P < 0.001$) linear dose–response relationship over power settings from 36 to 200 W, with 77 per cent of the variation in lesion diameter being accounted for by variation in power. The middle (solid) line in *Fig. 4* shows the estimated mean lesion diameter as a function of power. *Fig. 4* also shows the prediction interval of individual lesions (broken line) at a given wattage following a 3-min treatment. For instance, a 3-min treatment with 150 W had a 95 per cent chance of producing a volume of ablation 4 cm in diameter, with a 5 per cent chance that the lesion size produced would be greater than 6 cm in diameter.

Animals tolerated the procedure well. No evidence of infection, haemorrhage or bile leaks was noted in any of the lesions. Most pigs were mobile and feeding within 6 h of the procedure, and none exhibited signs of a systemic inflammatory response syndrome. All pigs scheduled to survive 1 week or longer gained weight and did not exhibit any signs of stress. No animal suffered microwave-related complications.

Discussion

This study shows that the UBMDTG microwave equipment is able to produce large-volume ablations of hepatic parenchyma from 3 to over 6 cm in diameter very rapidly, i.e. within 3 min. Lesion production was controllable and predictable, and the majority of lesions were spherical. Lesion geometry mimicked the microwave field, which radiated in an almost spherical fashion.

The new design of the applicator allowed increased penetration of the microwave field through the tissue throughout the treatment cycle. Increasing radiating efficiency resulted in a far greater proportion of the power being transmitted into the tissue rather than being dissipated in heating the applicator. This allowed the use of higher powers than has previously been possible with alternative microwave devices. Such devices are unable to utilize such high-power settings owing to reflection at the applicator–tissue interface during treatment[1,5]. The combination of increased radiation efficiency and higher power resulted in the induction of large ablated volumes of hepatic parenchyma.

Lesion size is determined by the radius from the applicator at which a cytotoxic temperature is reached. Since cytotoxicity is a function of time at temperature as well as the absolute temperature[10], lesion size is a function of time if all other variables are equal. However, if the temperature does not reach a certain critical level, cell death will not be achieved regardless of the exposure time. Currently no other ablative technique is able to deliver such large volumes of ablation in such a short time. Indeed, of the few ablative techniques that can produce such large ablation diameters with a single applicator insertion, the time required to do so is much greater and in some cases associated with significant morbidity[11–14].

The immediate findings of an inner blanched area surrounded by an outer, darker rim are in accordance with previous macroscopic findings[3,5,15]. This outer zone is a haemorrhagic region; the blanched zone and eventual lesion extend into this area. This lesion enlargement occurs as hepatocytes within this outer haemorrhagic zone experience smaller thermal doses and undergo death more slowly. The combination of thermal stress in conjunction with ischaemia from ruptured capillaries causes the cell death in this region seen at 24 h. Demarcation of the lesions was evident, especially from 24 h onwards, suggesting that there may be a definitive temperature at which irreparable hepatocyte damage occurs.

At all timepoints the microwave equipment behaved in a predictable, dose-dependent manner. This is important as safe and predictable treatment of liver tumours requires knowledge of the degree of tissue destruction and healing that occurs following ablation. The line of best fit does not go through the origin (*Fig. 4*) as there appears to be a non-linear relationship between lesion diameter and power at lower, and possibly higher, power levels. The dose–response curve does show some individual variation in diameter, although most of this is likely to be due to biological factors such as blood flow and liver lobe size. Large-volume microwave ablation of liver parenchyma was not associated with significant morbidity or mortality in this model. In contrast to published experience with cryotherapy, no cracking or haemorrhage was noted either during or following the microwave procedure[16]. As the microwave applicator is a single cylinder with no other moving parts, it does not require the placement of numerous energy sources or have the potential problems associated with retraction of multiple arrays[17,18].

This microwave equipment has the potential to alter treatment of patients with irresectable colorectal liver metastases or hepatocellular carcinomas. The current applicator is 6·8 mm in diameter, requiring placement at laparotomy, although a 5-mm flexible laparoscopic applicator has recently been developed. Work is in progress on a new 3-mm applicator suitable for percutaneous use, although this has required the use of alternative materials at the applicator tip. Computer models of its microwave field are being assessed. This may allow the treatment of high-risk patients by avoiding a laparotomy.

This new microwave equipment works in a safe, dose-dependent and efficacious manner. This development represents a significant advance in microwave ablation technology and may have great potential in the treatment of irresectable liver tumours.

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Original article

The surgeon as a prognostic factor after the introduction of total mesorectal excision in the treatment of rectal cancer

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Background: With conventional blunt surgical resection of rectal cancer, local recurrence rates are high and the individual surgeon putatively influences patient outcome. With total mesorectal excision (TME) local recurrence rates have been reduced and intersurgeon variability may be less important. The 'TME project' was a collaborative project that included surgical workshops in Stockholm between 1994 and 1997. The aim of this study was to assess the impact of the project on the practice of rectal cancer surgery in Stockholm and to analyse whether surgeon case volume and participation in the workshops influenced patient outcome.

Methods: All 652 patients who had an abdominal resection for rectal cancer in Stockholm between 1995 and 1997 were included. Outcome was compared in patients operated on by teams that included high-volume surgeons (more than 12 operations per year) with teams that included low-volume surgeons (12 operations or fewer per year), as well as between teams that including workshop participants and non-participants.

Results: Forty-six surgeons operated on the 652 patients. Five high-volume surgeons operated on 48 per cent of the patients. In these, outcome was significantly better than in patients treated by low-volume surgeons (local recurrence rate 4 *versus* 10 per cent ($P = 0.02$); rate of rectal cancer death 11 *versus* 18 per cent ($P = 0.007$)). Twenty-six surgeons were workshop participants and performed 93 per cent of the operations. Radiotherapy, TME and sphincter-preserving surgery were more common among patients treated by workshop participants.

Conclusion: The TME project has had an impact on rectal cancer surgical practice in Stockholm. Variability in patient outcome was mainly related to case volume, with better results obtained in patients treated by high-volume surgeons.

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Introduction

Rectal cancer surgery is technically difficult and outcome may be influenced by the individual surgeon as well as the entire team at the institution in which the patient is treated[1–6]. Conventional surgical techniques have usually been performed, using blunt dissection of the rectum and mesorectum with a high risk of tearing the mesorectum and presacral nerves. With this approach, the individual surgeon influences patient outcome in terms of postoperative morbidity and mortality, local recurrence rate and cancer-free survival[2–4]. Local recurrence rates after potentially curative surgery have varied between 5 and 40 per cent in different reports[7–12]. With the introduction of total mesorectal excision (TME) as a standardized, precise surgical technique very low local recurrence rates have been reported by individual institutions[13–16].

The TME teaching initiative in Stockholm, Sweden (the 'TME project'), a collaborative project of surgical workshops, demonstrated that TME surgery improved treatment results in a defined population of 1·9 million[8]. The local recurrence rate was significantly reduced and the cancer-specific survival was improved after the introduction of the TME concept to colorectal surgeons in Stockholm. In addition, the proportion of patients who underwent abdominoperineal resection was significantly reduced[8].

The aim of this study was to further assess the impact of the TME project on rectal cancer surgical practice in Stockholm, in particular to analyse whether surgeon case volume and surgeon participation in the workshops influenced patient outcome.

Patients and methods

Details on the TME project have been published previously[8]. In summary, three workshops were held at the Karolinska Hospital in Stockholm between 1994 and 1997. During 11 video-based live surgery sessions and two histopathology sessions, details of the TME technique and assessment of specimen circumferential margin involvement were demonstrated and discussed. Ample time was allocated for discussion around prerecorded videos that described details of the TME technique. The workshops specifically encouraged voluntary self-selection and specialization to meet the evident surgical challenge posed by the TME technique.

In Sweden, the reporting of a new cancer case is compulsory for the treating physician and the responsible pathologist. In addition, since 1995, clinical data on all patients with rectal cancer treated in the Stockholm area (1·9 million inhabitants) have been reported prospectively to the Regional Oncologic Centre by the responsible surgeon and pathologist. The reports include details of patient characteristics, adjuvant treatment, type of surgery including whether TME was performed, postoperative morbidity and mortality, and follow-up data on local recurrence, distant metastases and cause of death.

All patients who had an abdominal resection for rectal adenocarcinoma in Stockholm between 1995 and 1997 were included in the present study. The anastomotic leak rate, reoperations and postoperative mortality (within 30 days of surgery) were analysed in all patients. Other surgical complications were not analysed, as validated data were not available. The incidence of local recurrence and distant metastasis, as well as cancer-specific survival were analysed in patients operated with curative intent. Surgery was defined as curative if the surgeon reported a complete tumour resection, without distant metastases, and if the pathologist reported tumour-free margins of the specimen. Staging of the tumours was done according to the tumour node metastasis system. Local recurrence was defined as a recurrence within the pelvis, excluding metastases in the ovaries. Death in the presence of local recurrence, or distant spread before or at time of death, was regarded as a rectal cancer-specific death. Death with no evidence of recurrent disease was regarded as a non-rectal cancer death.

To identify the individual surgeons performing the operations during the study period, operative notes on all patients were reviewed and each individual, board-certified surgeon participating at the operation was recorded. Trainees participating under supervision were therefore excluded from the study.

The number of operations performed by each surgeon was calculated by counting the number of patients in whose operation the surgeon had actively participated. One or two surgeons were recorded for each patient; if two board-certified surgeons participated at one operation, both were considered to have performed the operation and it was counted as one operation for each of them. To calculate the mean annual number of operations for each surgeon the total number of operations performed by that surgeon was divided by the number of years of the study during which he or she was active as an operating surgeon. A surgeon performing more than a mean of 12 rectal cancer operations annually was classified as a high-volume surgeon. A surgeon who performed on average 12 or fewer operations annually was classified as a low-volume surgeon. This categorization was made in accordance with Hermanek *et al.*[17], who estimated that a volume of between one and two rectal cancer operations per month was necessary to achieve favourable long-term results.

The identified surgeons were matched against the participation lists from the workshops. A surgeon who had attended the workshops at least once was defined as a workshop participant. A surgeon who had not attended a workshop was defined as a workshop non-participant.

Patients were classified according to whether they were operated on by a high-volume or low-volume surgeon. If one or both of the surgeons were high-volume surgeons the patient was classified as having had the operation done by a high-volume team. If none of the surgeons were high-volume surgeons the patient was considered to have had the operation done by a low-volume team.

Each patient was also classified as having been operated on by a workshop participant or by a workshop non-participant. If one or both of the surgeons were workshop participants the patient was classified as having had the operation performed by a workshop participant team. If none of the surgeons were workshop participants the patient was considered to have had the operation performed by a workshop non-participant team.

Follow-up data on all patients were available up to December 1999. Two patients were lost to follow-up owing to emigration. The median follow-up time was 41 (range 24–59) months.

Statistical analysis

The total number of local recurrences and distant metastases were analysed. Patients were considered to be at risk of the studied event until death, emigration or to the end of follow-up (closing date 31 December 1999).

Comparisons of proportions were made using the χ^2 test. The hazard rate ratios were analysed using Cox proportional hazards regression model. In multivariate analyses adjustment was made for age, sex, tumour stage, tumour level above the anus, radiotherapy and TME procedure. Comparisons of the hazard rates of local recurrence, distant metastases and rectal cancer death were made using the likelihood ratio test.

Results

A total of 652 patients with rectal cancer had an abdominal resection between 1995 and 1997. The patients were operated on by 46 surgeons. The total number of rectal cancer operations performed by each surgeon, alone or as one of two surgeons, varied between one and 143 during the study period. Twenty-four surgeons performed between one and 17 operations, 17 performed 20–36 operations and five performed 46–143 operations. The mean annual number of operations performed by each surgeon varied from one to 48. Forty-one low-volume surgeons performed 12 or fewer operations per year (one or less per month) and five high-volume surgeons averaged more than 12 operations per year (more than one per month). Twenty-six of the 46 surgeons had participated in the TME workshops and were classified as workshop participants.

Patient characteristics are shown in *Table 1*. Preoperative radiotherapy was given to 369 patients (57 per cent) and 450 patients (69 per cent) had a sphincter-saving procedure. A TME procedure (defined as removal of the rectum with the entire mesorectum down to the pelvic floor) was reported by the surgeon in 531 patients (81 per cent). Curative surgery was performed in 522 patients (80 per cent). The distribution of age and sex, the proportion receiving preoperative radiotherapy and the type of surgery performed was similar for all patients and those operated on with curative intent. The proportion of stage I and II tumours was higher in the curatively operated group than the whole patient group.

Clinical characteristics for patients operated on by the high- and low-volume teams are listed in *Table 1*. The distribution of sex, age and tumour stage was similar between the groups. Sixty per cent of patients operated on by the high-volume teams received preoperative radiotherapy compared with 53 per cent of patients operated on by the low-volume teams ($P = 0{\cdot}05$). The high-volume teams performed TME resections in a higher proportion of patients than the low-volume teams (87 *versus* 76 per cent; $P < 0{\cdot}001$). No significant differences were seen in the proportion of curative procedures, anterior resections or crude rates of postoperative mortality (3 *versus* 4 per cent), anastomotic leaks (10 *versus* 11 per cent) or reoperations (12 *versus* 10 per cent) between high- and low-volume teams.

Table 1 Clinical characteristics of patients who had abdominal surgery with resection of rectal cancer, grouped according to operation by high- and low-volume surgical teams, and workshop participant and non-participant teams

	All patients (*n* = 652)	Curative surgery (*n* = 522)	High-volume team (*n* = 316)	Low-volume team (*n* = 336)	Workshop participant team (*n* = 606)	Workshop non-participant team (*n* = 46)
Demography						
Male	367 (56)	288 (55)	186 (59)	181 (54)	351 (58)	16 (35)
Female	285 (44)	234 (45)	130 (41)	155 (46)	255 (42)	30 (65)
Median (range) age (years)	71 (28–93)	69 (28–93)	70 (34–90)	72 (28–93)	71 (28–91)	74 (44–93)
Preoperative radiotherapy	369 (57)	306 (59)	191 (60)	178 (53)	351 (58)	18 (39)
Tumour stage*						
I	175 (27)	169 (32)	82 (26)	93 (28)	163 (27)	12 (26)
II	214 (33)	200 (38)	99 (31)	115 (34)	199 (33)	15 (33)
III	185 (28)	153 (29)	93 (29)	92 (27)	171 (28)	14 (30)
IV	76 (12)	0 (0)	42 (13)	34 (10)	71 (12)	5 (11)
Missing	2 (0)	0 (0)	0 (0)	2 (1)	2 (0)	0 (0)
Type of surgery						
Anterior resection	450 (69)	372 (71)	228 (72)	222 (66)	427 (70)	23 (50)
Abdominoperineal resection	162 (25)	132 (25)	61 (19)	101 (30)	144 (24)	18 (39)
Hartmann's procedure	40 (6)	18 (3)	27 (9)	13 (4)	35 (6)	5 (11)
TME procedure	531 (81)	442 (85)	274 (87)	257 (76)	502 (83)	29 (63)
Tumour distance from the anus (cm)						
0–5	191 (29)	157 (30)	91 (29)	100 (30)	179 (30)	12 (26)
6–10	234 (36)	194 (37)	112 (35)	122 (36)	214 (35)	20 (43)
> 10	216 (33)	163 (31)	105 (33)	111 (33)	203 (33)	13 (28)
Missing	11 (2)	8 (2)	8 (3)	3 (1)	10 (2)	1 (2)

Values in parentheses are percentages unless otherwise stated. *Tumour node metastasis classification. TME, total mesorectal excision

Forty-eight per cent of the patients were operated on by five high-volume teams, all of whom had participated in the TME workshops. Among patients operated on by the five high-volume teams no significant differences were seen regarding postoperative morbidity and mortality rates, rate of local recurrence, distant metastases or rectal cancer death (data not shown). The crude rate of local recurrence after curative surgery was significantly lower in patients operated on by high-volume teams than in those treated by low-volume teams (4 *versus* 10 per cent) (*Table 2*). If the teams were divided into further subgroups, teams that undertook six or fewer rectal cancer operations per year had a recurrence rate of 16 per cent compared with 9 per cent ($P = 0{\cdot}02$) and 4 per cent ($P = 0{\cdot}005$) for those who did between seven and 12 operations per year and more than 12 operations per year respectively. The crude rates of distant metastasis were not significantly different but fewer patients died from rectal cancer in the group operated on by high-volume teams (11 per cent) than in the group operated on by low-volume teams (18 per cent). After adjustment for confounding factors in the multivariate analysis, there was still an increased risk of local recurrence and rectal cancer death in patients treated by low-volume teams. No differences were found with respect to the studied endpoints when the three study years were analysed separately (data not shown).

In 606 patients (93 per cent) the operation was performed by a workshop participant team (*Table 1*). Only 46 patients (7 per cent) were treated by a team of workshop non-participants. In 295 patients (45 per cent) the team performing the operation included one workshop participant and in 311 patients (48 per cent) the team included two surgeons who were workshop participants.

The rate of curative surgery was similar in patients treated by workshop participant and non-participant teams. However, patient characteristics for those operated on by workshop participant teams differed from those operated on by workshop non-participant teams in that the proportion of women was lower (42 *versus* 65 per cent; $P < 0{\cdot}005$), more patients received radiotherapy (58 *versus* 39 per cent; $P = 0{\cdot}01$) and more patients had a sphincter-preserving procedure (70 *versus* 50 per cent; $P < 0{\cdot}005$). Sixteen patients (3 per cent) had an emergency operation carried out by a workshop participant team compared with two patients (4 per cent) in the group operated on by workshop non-participant teams (*P* not significant). A TME resection was reported in 502 patients (83 per cent) treated by a workshop participant team and in 29 patients (63 per cent) treated by workshop non-participant teams ($P < 0{\cdot}001$). Tumour stage distribution was similar between the two groups.

The proportion of anastomotic leaks (10 *versus* 9 per cent) and reoperations (11 *versus* 13 per cent) was similar between workshop participant and non-participant teams. No difference was seen in postoperative mortality rate between the two groups of patients.

The crude local recurrence rate after curative surgery was 6 and 13 per cent in patients operated on by workshop participant and non-participant teams respectively (*P* not significant). The crude rate of distant metastasis was 17 *versus* 24 per cent, while 14 *versus* 18 per cent died from rectal cancer (*P* not significant).

Discussion

During the study period between 1995 and 1997 more than 80 per cent of patients who had an abdominal resection for rectal cancer in Stockholm were operated on with the TME technique. Ninety-three per cent of the patients were operated on by 26 TME workshop participants and five of these were classified as high-volume surgeons who operated on almost 50 per cent of the patients. The mean annual number of rectal cancer operations performed by each surgeon varied between one and 48, and the total number varied between one and 143 in 3 years. In comparison, within the two Stockholm Radiotherapy Trials (1980–

Table 2 Local recurrence, distant metastasis and rectal cancer death in curatively operated patients, according to operation by high- or low-volume teams

	High-volume team*	Low-volume team*	Hazard ratio†	*P*‡
Mean no. of operations per year	> 12	0–12		
Curative surgery	245 (78)	277 (82)		
Median (range) follow-up (months)	41 (24–59)	43 (24–59)		
Local recurrence	9 (4)	27 (10)	2·4 (1·2, 5·6)	0·02
Distant metastasis	39 (16)	54 (19)	1·2 (0·8, 1·9)	0·33
Rectal cancer death	26 (11)	51 (18)	1·9 (1·2, 3·1)	0·007

Values in parentheses are *percentages unless otherwise stated or †95 per cent confidence intervals. Hazard ratios (low- *versus* high-volume team) were adjusted for age, sex, tumour stage, tumour level above anus, radiotherapy treatment and total mesorectal excision operation. ‡Log rank test

1993) 149 different surgeons operated on 1372 patients, the mean annual number of operations performed by each surgeon varied between one and nine, and only 11 surgeons performed more than 25 operations (range 35–87) during the study period of 12 years[1]. The TME project is therefore likely to have had an impact on rectal cancer surgical practice in Stockholm. Furthermore, the workshop has encouraged voluntary self-selection and specialization which has developed in a short period of time. In addition, the present study shows that patients operated on by teams including high-volume surgeons had a significantly better outcome.

TME and sphincter-saving procedures were performed more often and radiotherapy was more commonly used in patients treated by workshop participant teams than in patients treated by workshop non-participant teams, despite a similar tumour stage distribution in the two groups of patients. The local recurrence rate and the rate of rectal cancer deaths were lower in patients operated on by workshop participants although the differences were not statistically significant, which could be related to the small number of patients treated by workshop non-participant teams.

Case volume has been suggested as an indicator of surgical quality for individual surgeons[2,3,17]. The present results are in accord with this hypothesis. In the present study patient outcome was significantly influenced by the surgeon's case volume. The local control and cancer-specific survival was significantly better in patients operated on by high-volume than low-volume teams, while the risk of postoperative morbidity and death was similar between the two groups of patients. There were no significant differences in treatment results related to the individual surgeon within the group of five high-volume teams.

In the Stockholm Radiotherapy Trials, conventional blunt surgical techniques were typically used and case volume did not significantly influence outcome[1]. TME in rectal cancer is a difficult and technically demanding operation. A high case volume appears to be important in order to achieve good results. The five high-volume surgeons had participated in all three workshops and had a continuous high case volume during the study period.

Other studies have addressed surgeon case volume in cancer surgery[18–22]. Some focused on postoperative mortality and morbidity, and demonstrated a surgeon-related variability, mainly related to case volume[19,21]. However, other studies that addressed short-term outcome showed no association with case volume[4,22]. Similarly, in the present study no differences were seen in postoperative mortality and morbidity rates between high- and low-volume teams or workshop participants and non-participants. In addition, Porter *et al.*[3] showed that outcome was improved both with colorectal subspecialty surgical training and a higher frequency of rectal cancer surgery. In the Stockholm trials, patients operated on by surgeons who had been certified specialists for at least 10 years also had a lower risk of local recurrence and death from rectal cancer than patients operated on by surgeons with less experience[1].

In this study a board-certified surgeon was considered to have performed the operation when he or she had actively participated at surgery. A maximum of two surgeons was recorded for each patient. It may be argued that only one surgeon should have been recorded for each operation. However, the authors believe that when two board-certified surgeons actively participate both of them increase their experience and skill. For example, if a team of two surgeons performs 50 TME resections and only the senior surgeon is recorded as the operating surgeon, the second surgeon would be regarded as a low-volume surgeon when he or she performed the 51st TME resection together with another surgeon. This would not reflect the actual experience of the second surgeon and it was considered preferable to consider each surgeon as having performed and gained experience from each resection.

Another potential problem in this study was that the total experience of the surgeon and the team, i.e. the volume of rectal cancer operations before the study period, was not taken into account. However, all surgeons had several years of experience after board certification and there were no obvious differences with regard to experience between workshop participants and non-participants, and between high-volume and low-volume surgeons (data not shown).

The fact that outcome was significantly better in patients treated by high-volume surgeons is probably explained by the greater experience in TME resections *per se* within this group of surgeons. High-volume surgeons performed TME and used radiotherapy more often than low-volume surgeons; however, after adjustment for these variables in the multivariate analysis the differences in outcome concerning local recurrence and rectal cancer death were significantly better in patients operated on by high-volume surgeons. There may, of course, be other factors influencing outcome and related to high-volume surgeons, which have not been adjusted for. In addition to high case volume, outcome may be influenced by the knowledge of 'the TME concept' or 'specimen-orientated surgery'. This includes proper preoperative staging, the use of preoperative radiotherapy, meticulous surgical dissection with rectal wash-out and pathological evaluation of the circumferential resection margin.

In conclusion, the TME project apparently had a significant impact on rectal cancer surgical practice and patient management in a defined population of 1·9 million in Stockholm. Variability in patient outcome was mainly related to case volume, with better results obtained in patients treated by high-volume surgeons who had participated in the workshops. To further improve results in rectal cancer all patients should be offered care by a well educated, high-volume team that pays meticulous attention to detail. With today's standardized preoperative staging, radiotherapy techniques and surgical skill, low rates of local recurrence should be achieved.

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Original article

Experience of surgery for rectal cancer with total mesorectal excision in a general surgical practice

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Background: Results from specialist centres have shown that total mesorectal excision (TME) produces excellent control of local disease in patients with carcinoma of the rectum.

Methods: The results of TME were reviewed in a surgical practice in which patients with rectal cancer comprised 1 per cent of the total caseload and mean case numbers were less than 15 each year.

Results: Eighty-two consecutive patients underwent rectal excision with TME over a 72-month period (68 anterior resection, eight abdominoperineal excision and six Hartmann's procedure). Sixty-nine operations were deemed 'curative' at the time of surgery. Anastomotic leak occurred in two (3 per cent) of 68 patients, both of whom recovered without additional surgery. There were two local recurrences (3 per cent) among 69 patients who underwent 'curative' surgery. At a median follow-up of 190 weeks, the survival rate for Dukes' stage A, B, C and 'D' was 100, 83, 68 and 18 per cent respectively.

Conclusion: Outcome as measured by perioperative morbidity and local disease control achieved in a surgical practice with a broad case mix and relatively low annual case volume was comparable to that from larger centres. Appropriate surgical training and attention to technical detail may be as important as case volume in determining outcome after surgery for rectal cancer.

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Introduction

Total mesorectal excision (TME) has become the 'gold standard' in rectal cancer surgery[1,2]. All major published series have demonstrated low rates of local recurrence, good postoperative genitourinary function and a high rate of sphincter preservation[1,3–6]. The introduction and application of TME has also led to some controversies. Concerns have been expressed regarding the potential for selection bias in results that have emanated from specialist units, the reproducibility of results in general surgical practice, the occurrence of intersurgeon variability and the impact of surgical volume and case mix on outcome[7–11]. It is currently unclear what influence these individual variables have. Elimination of the potential for selection bias is not possible in specialist centres and it is difficult to control for the influence of surgical training and experience when discussing case volume and case mix issues. It is essential for proper organization of rectal cancer surgical services and to ensure the quality of healthcare delivery to patients that there is clarity regarding these issues. The objective of this study was evaluate whether a general surgeon with formal training in the technique of TME can achieve results that compare with internationally accepted best practice, in the setting of a non-specialist practice (i.e. that of a general surgeon with an interest in surgery of the gastrointestinal tract).

Patients and methods

Patients

Eighty-two consecutive patients underwent surgery for rectal cancer (tumours located within 15 cm of the anal verge), between August 1993 and July 1999, and are the subject of this report. Seventy-nine patients (96 per cent) were referred directly from a primary care physician. Seventy-six patients had an elective procedure and six had emergency surgery (four for large bowel obstruction and two for perforation, which in one patient was sustained during a preoperative contrast study). Forty patients were American Society of Anesthesiologists (ASA) grade I, 37 ASA II and five ASA III. Over the study period, three patients were refused surgery because of co-morbid medical conditions which, in the opinion of the surgeon or anaesthetist, precluded safe general anaesthesia. The mean age of the patients was 63·4 (range 38–81) years.

The female : male ratio was 1·14 : 1. Data concerning clinicopathological staging and postoperative course were collected prospectively.

Operative technique

All patients were operated on by a single surgeon (O.J.McA.), or under his direct supervision, in a single institution. The rectal dissection for TME was conducted in standard fashion with sharp dissection in the appropriate anatomical plane with total excision of the mesorectum[2,12]. The splenic flexure and transverse colon were mobilized in all patients. At anterior resection a double-stapling technique was employed and colonic continuity was re-established by colorectal or coloanal anastomosis using a circular stapling device (AutoSuture, US Surgical, Norwalk, Connecticut, USA). In all patients a hand-placed purse-string suture of 2/0 polypropylene through all layers was used to contain the anvil of the stapling device in the proximal limb of the anastomosis before application of the staples. At completion of the anastomosis, the bowel edges excised by the circular stapler ('doughnuts') were examined for completeness. In all patients the neorectum was tested for anastomotic air leak. A suction drain was placed in the pelvis and a diverting stoma was performed according to the criteria set out in *Table 1*.

Patients who had abdominoperineal excision had dissection down to, and between, the levator ani muscle completed during the abdominal component of the procedure. Only mobilization and dissection of the anal canal–sphincter complex was performed though the perineal incision. All six patients who underwent emergency surgery had a Hartmann's procedure with cross-stapling of the distal rectum, without re-establishment of colonic continuity on the basis of the patients' compromised overall preoperative status. All patients had intraoperative placement of a Bonano® suprapubic urinary catheter (Becton Dickenson, Franklin Lakes, New Jersey, USA).

Specimens were analysed in a standard manner. Serial sections were performed as well as analysis of the inked circumferential margin, grading and Dukes' staging. As many lymph nodes as possible were dissected from the mesorectum for histological analysis.

Table 1 Criteria used for formation of a diverting stoma

Age > 75 years
Faecal spillage
Doughnuts not intact
Air leak on testing
Surgeon's discretion

Following surgery patients attended the outpatient clinic every 3 months for 2 years, 6 monthly up to 5 years and yearly thereafter. Patients were examined clinically and serum carcinoembryonic antigen levels were estimated at each visit. Liver ultrasonography or computed tomography was performed every 6 months and colonoscopy was performed at 1 and 3 years after operation.

Chemotherapy and radiotherapy

Patients with Dukes' C tumours were counselled regarding the potential benefits and morbidity of adjuvant radiotherapy. Eleven opted to have postoperative adjuvant radiotherapy (40 Gy). The considerable distance from this centre to the nearest radiotherapy centre (over 210 km) may explain the poor uptake of radiotherapy. Three others had radiotherapy because their resection margins contained macroscopic or microscopic foci of tumour. Two patients had preoperative neoadjuvant radiotherapy for locally advanced disease on the basis of preoperative staging investigations.

All patients aged less than 70 years with node-positive disease were given chemotherapy with 5-fluorouracil and leucovorin for 12 months.

Statistical analysis

Descriptive statistics (number, median, first and third quartiles) were calculated for level of the tumour, Dukes' stage and type of operation (anterior resection, abdominoperineal excision). Data were compared using non-parametric Wilcoxon tests. Cross-tabulations of categorical variables were produced, and the number of observations and the row and column percentages calculated. Frequencies were then compared formally with Fisher's exact test. For each patient, the survival time was calculated as the time from operation to the date on which the records were reviewed. Patients who were alive at the time had censored times to death. The survival rates and associated statistics were estimated using Kaplan–Meier methodology. These statistics and all other analyses were calculated using SAS®/STAT (SAS® version 6.12; SAS Institute, Cary, North Carolina, USA) by an independent professional statistician. Values are presented as mean(s.e.m.) as the data were distributed symmetrically.

Results

Sixty-eight patients underwent anterior resection (21 with a covering transverse loop colostomy), while eight patients whose tumour involved the sphincter had abdominoperineal resection. Six patients had proctectomy and end-colostomy with a cross-stapled rectal stump (Hartmann's). Sixty-nine resections were deemed curative at the time of surgery (no residual local or distant disease on inspection and palpation following resection). Of patients whose operation was deemed non-curative at the time of surgery, all subsequently had histologically proven local ($n = 2$), metastatic ($n = 10$) or local and metastatic ($n = 1$) residual disease.

The 30-day mortality rate was 4 per cent (myocardial infarction, one patient; respiratory failure secondary to an exacerbation of pre-existing chronic obstructive airway disease, one; stroke, one). In-hospital morbidity included pulmonary atelectesis (12 patients), superficial wound infection (six), pneumonia (one) and failure to void urine on first trial of suprapubic catheter clamping (one). One patient had an intraoperative blood transfusion, while 11 required a blood transfusion after operation (mean transfusion for these patients was 2·4 units). Six patients were treated with antibiotics after operation (other than the routine prophylactic dose), two for culture-positive urinary tract infection, one for pneumonia, one for an upper respiratory tract infection and two because of an anastomotic leak. The latter became pyrexic on the fifth day after operation and had signs of peritoneal irritation within the lower abdomen. The anastomotic leaks were confirmed by contrast studies. Both patients had a diverting loop colostomy constructed at the time of initial surgery, did not require further surgical intervention and went on to make a full recovery. Two patients did not undergo closure of a diverting stoma; one died following a stroke before reversal and the other refused closure surgery. There was no case of long-term urinary dysfunction.

Histological evaluation showed that 15 patients had Dukes' A, 26 Dukes' B and 30 Dukes' C disease. Eleven patients had liver metastases (Dukes' 'D'). There were five local recurrences at a median follow-up of 190 weeks. Three of these were in patients whose operation was not initially considered curative (one patient had a perforated tumour, another had advanced local disease with positive mesenteric margins and the other sustained an iatrogenic perforation during operation). There were two local recurrences during follow-up among 69 resections that were considered curative (3 per cent), in one patient with Dukes' B and one with Dukes' C disease. Both patients had received adjuvant (postoperative) radiotherapy.

Twenty-nine patients had tumours within 5 cm of the dentate line, 28 between 6 and 10 cm away, and 25 between 11 and 15 cm away. Mean age, sex and distance of the tumour from the dentate line did not differ between patients whose operation was deemed curative ($P = 0·83$, $P = 0·45$ and $P = 0·38$ respectively). The proportion of patients whose resection margins were shown histologically to contain tumour differed between those whose operation was deemed curative and non-curative ($P = 0·03$). When the tumour extended to the edge of the surgical specimen only 50 per cent of the operations were thought to be curative, while 94 per cent of operations were deemed curative when the tumour was subsequently shown on histological analysis to have been excised completely.

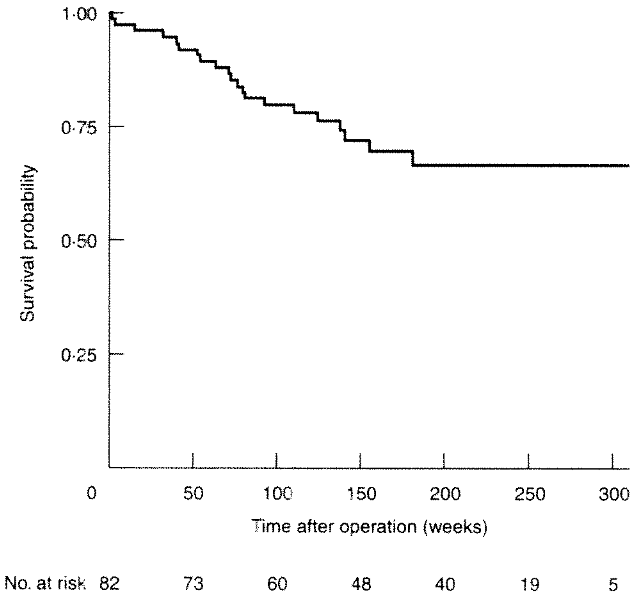

Fig. 1 Kaplan–Meier survival curve for all 82 patients in the series

When patients whose operation was deemed curative at laparotomy were examined in isolation, the Dukes' stage of the tumour was a significant determinant of survival ($P < 0·001$). None of the patients with Dukes' A disease died, while 17, 32 and 82 per cent of those with Dukes' B, C and 'D' tumours respectively had died at a median follow-up of 190 weeks. The overall 5-year survival rate (all stages) was 67 per cent (*Fig. 1*). After curative operations a higher proportion of patients who subsequently died underwent an abdominoperineal resection or Hartmann's procedure (36 per cent) than anterior resection (22 per cent) ($P = 0·015$) (*Table 2*).

Over the time period of this study, a mean(s.e.m.) of 973(57) patients annually underwent surgical procedures other than colorectal operations. Colorectal procedures comprised 3(1) per cent of the total annual caseload, while rectal cancer surgery accounted for 1(0) per cent of operations annually.

Discussion

In spite of advances in the understanding and treatment of colorectal adenocarcinoma, disease recurrence after

Table 2 Factors analysed for association with death (curative operations only)

	*P**
Sex	0·19
Age	0·49
Tumour level	0·73
Operation type	0·015†
Adjuvant therapy	0·01‡
Dukes' stage	< 0·001§
Involvement of margins	1·0
Local recurrence	1·0
Distant recurrence	< 0·001¶

*Fisher's exact test. †Patients who had anterior resection were significantly more likely to survive than those who had an abdominoperineal resection or Hartmann's procedure; ‡patients who required adjuvant therapy were significantly less likely to survive; §increasing Dukes' stage significantly correlated with increased likelihood of death; ¶distant recurrence was significantly associated with increased mortality

apparently curative surgery remains a significant problem. In the UK there are in excess of 28 000 new diagnoses annually and approximately 19 000 deaths[13]. The patterns of recurrence differ for colonic and rectal tumours; disseminated disease is more common in the former, while rectal cancers tend to recur locally[14–16]. The reported incidence of local recurrence following curative resection of rectal cancers ranges between 3 per cent[17] and 32 per cent[18] with a median of over 15 per cent[19]. The 5-year survival rate following local recurrence is 5 per cent with median survival of 7 months[20]. Because successful palliation of local recurrence is difficult to achieve, it has frequently been suggested that reducing local recurrence is as important as increasing overall survival in patients with rectal cancer[21,22].

The application of TME in rectal cancer surgery has produced consistently low rates of local disease recurrence[22,23]. However, the potential for perioperative morbidity and the variations in reported data have led to discussions on intersurgeon variability, surgical case mix and surgical specialization since TME has been popularized[10,24,25]. There has been some concern that increased morbidity and mortality rates may occur, particularly as a result of increased rates of anastomotic leakage. These have approximated 10 per cent in many series and this has led to a policy of increased use of diverting stomas[26,27]. The authors have maintained a policy of selective use of loop stomas (31 per cent in this study) using the criteria outlined, but the introduction of colonic J pouches in selected patients may lead to increased use. Of the two anastomotic leaks in the series, both occurred in patients with diverting stomas. Postoperative morbidity was otherwise limited and the mortality rate was 4 per cent. These results compare well with those of other series.

Patients who underwent abdominoperineal resection or Hartmann's procedure had a significantly worse outcome than those who had anterior resection. This was also described by Heald *et al.*[21], who hypothesized that this may be related to tumour implantation in an abdominoperineal wound. This does not occur in an anterior resection. Alternatively the absence of a definable mesorectum below 3–4 cm from the anal verge may remove an important safety factor. Heald *et al.* also questioned whether all abdominoperineal resections should be preceded by radiotherapy. This question remains unanswered.

As regards restoration of continuity, eight of the 82 patients were unsuitable for sphincter-preserving surgery (tumours involving sphincter). Of the remainder, 66 of the 68 patients who had anterior resection had continence restored, but none of the patients with a cross-stapled rectal stump was reanastomosed. In two of these patients an initial anastomosis was not attempted because of pelvic sepsis at the time of original surgery, but in the other four patients a more suitable approach may have been construction of a temporary diverting stoma initially, followed by definitive surgery later. With this approach, it is possible that four patients could have been spared a permanent stoma.

An important issue raised by this study is whether or not low rates of local recurrence can be achieved outwith specialist units[1,3–6]. Because of the nature of such units, it is possible that referral patterns select patients who are more likely to benefit from their expertise and so preselection bias could inadvertently have been a factor in the superior results achieved with TME. The data presented in this report refute such a suggestion, as comparable results were achieved in a practice with a predominantly secondary rather than tertiary referral pattern (i.e. there was no preselection bias). One might suggest that a local recurrence rate of 3 per cent was not accurate as 13 patients were excluded from the analysis. Of these 13 patients, 11 had liver metastases at the time of surgery, one patient had a perforated tumour, another had advanced local disease with positive mesenteric margins and the other sustained an iatrogenic perforation during operation. While some caution must be exercised in interpretation of the data, as the median follow-up for the patients presented was 190 weeks, it has been shown that at least 80 per cent of local recurrence becomes apparent by 104 weeks[1]. The projected local recurrence rate at 5 years for the present patients should remain below 4 per cent.

Much discussion has been centred on the appropriate delivery of surgical services, with the suggestion that rectal cancer surgery should be performed in dedicated units by specialist surgeons[10,24,25]. This has obvious implications for organization of services, especially emergency surgery, and for surgical training as the treatment of a relatively common cancer may no longer be the remit of many surgeons, surgical trainers and surgical trainees. Two critical questions are central to these discussions; first, how does surgical case mix affect results and, second, what is the critical annual volume of patients required to maintain expertise? To date, these questions have not been answered clearly, because the individual surgeon's training and experience in TME surgery has not been controlled for[10,24,25]. The present report helps to answer these questions as surgical training was not at issue, since the senior surgeon (O.J.McA.) was trained at the unit where TME was developed[21]. In a non-specialist practice where colorectal cancer formed 3 per cent of the annual workload and rectal cancer accounted for 1 per cent, a local recurrence rate of 3 per cent was achieved. Other reports have suggested that results are compromised by a broad case mix[28], but it is clear from the data presented that adequate training may offset this. It has recently been suggested that the proportion of patients undergoing abdominoperineal resection with a permanent stoma was lower in hospitals with a high caseload[29]. This study did not examine the results of individual surgeons, only institutions, and the rate of permanent stoma formation in the present study (17 per cent) compares favourably.

Recently, the role of surgical training in TME surgery has been endorsed, following the introduction of TME to surgeons in Stockholm in 1994. By means of video teaching and histological presentation this led to a reduction in the local recurrence rate to 6 per cent[1]. Taken together with the data presented here, appropriate training in the technique of TME is the essential factor, and absolute specialization is not necessary to produce good results. Undoubtedly a critical number of patients is required to maintain standards, and in the authors' experience an average caseload of approximately 14 per year did not compromise results.

TME may be performed successfully by non-specialist surgeons who have been trained appropriately in the technique, even in the presence of a varied caseload and an annual rectal cancer case volume as low as 14. As training rather than volume predicts outcome, rectal cancer surgery need not be limited to specialist units as long as results are strictly audited.

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Endoscopic resection of large sessile colonic polyps by specialist and non-specialist endoscopists

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Background: Patients with large sessile colonic polyps for which endoscopic resection might be feasible are often referred directly for operation. The aim of this study was to determine the outcome of patients with such polyps diagnosed by specialist and non-specialist colonoscopists.

Methods: Patients with large (greater than 2 cm) sessile polyps detected at colonoscopy from January 1995 to July 2000 were identified. Resection technique and clinical and endoscopic outcomes were recorded. Two of the colonoscopists (B.P.S., C.B.W.) are recognized as specialists.

Results: Some 130 polyps were identified, 100 detected by either of two specialist endoscopists (including 14 cancers) and 30 by 14 non-specialist endoscopists (including ten cancers). Endoscopic resection of benign polyps was attempted by experts in 80 (93 per cent) of 86 cases and by non-experts in 15 of 20 cases ($P = 0{\cdot}03$), with successful management by endoscopy alone in 61 (76 per cent) of 80 and in six of 15 cases respectively ($P = 0{\cdot}01$). Complications occurred following three polypectomies performed by an expert (bleeding, two; pain, one) and one by a non-expert (bleeding). The estimated end-cost of management by specialists was less than half of that by non-specialists.

Conclusion: Endoscopic resection of large sessile colonic polyps is feasible in the majority of patients and should be considered as first-line therapy. Prompt referral to a specialist endoscopist may improve outcomes by avoiding operation or enabling complete excision at a single endoscopy session.

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Background

Large sessile colonic polyps account for only a small proportion of colonic adenomas overall but are clinically important because of their propensity for malignant change[1]. Endoscopic resection became feasible in the 1970s with the development of piecemeal resection[2] and submucosal saline injection techniques[3,4]. Several case series have reported on the effectiveness and safety of endoscopic resection[5–7], but in centres with no special interest in colonoscopy techniques patients with large sessile lesions may still be referred directly for surgical resection. Reluctance to pursue endoscopic resection may result from lack of endoscopic expertise and concern at delaying definitive therapy for a polyp that may already contain undiagnosed malignancy. However, this practice exposes the patient to the risk of surgical morbidity and is likely to be less cost-effective than referral for a second endoscopic opinion.

A retrospective review was conducted of the outcomes of patients with large sessile colonic polyps diagnosed at colonoscopy over a 67-month period. The aim was to assess the results of endoscopic management by both specialist and non-specialist colonoscopists.

Patients and methods

Patients with large (greater than 2 cm) sessile polyps diagnosed during colonoscopy at this endoscopy unit from January 1995 to July 2000 were identified by a search of the computerized endoscopy database. The unit serves a local population of 330 000 as well as tertiary referrals to the ten colorectal surgeons and physicians and two specialist colonoscopists. During the study period a total of 12 715 colonoscopies was performed. The specialists performed five or six colonoscopy sessions per week (more than 800 colonoscopies per annum) including tertiary referrals of difficult colonoscopy cases. Non-specialists performed one or two sessions per week (fewer than 200 colonoscopies per annum).

Bowel preparation for colonoscopy entailed purgation with magnesium citrate (two 29·5 g sachets) and senna (weight 13 g), as well as dietary restriction for 2 days. Procedures were performed as day cases using conscious sedation, which was achieved by titrating intravenous boluses of midazolam and pethidine. Both specialist endoscopists also routinely administered intravenous hyoscine butylbromide (Buscopan®; Boehringer Ingelheim, Bracknell, UK) 20–40 mg, unless contraindicated. Procedures were performed with Olympus video-colonoscopes (CF200HL, CF230HL and IT200L; Olympus Optical, Tokyo, Japan). Olympus standard and mini-snares and Wilson–Cook injection needles (Bloomington, Indiana, USA) were used to perform snare resection and submucosal injection (using sterile normal saline). Diathermy settings were standardized to a 15-W coagulating current for all polypectomies.

Endoscopic and surgical records, case notes and pathology reports for each patient were examined. Polyps subsequently confirmed to contain advanced cancer (stage higher than T1) were included in the analysis only if sessile morphology had been noted and endoscopic resection attempted. Polyp size (often by comparison with open biopsy forceps) and morphology were generally estimated and recorded in the report by the endoscopist. Where this information was not available, dimensions were obtained from the histopathological record. Details of polyp location, initial resection technique and any operations or complications were obtained wherever possible. For patients no longer followed at this institution, the general practitioner or referring consultant was contacted. Polyps located in the distal 5 cm of the rectum were excluded, as proximity to the dentate line makes them most amenable to transanal surgical resection. Six patients with sessile lesions associated with ulcerative colitis (dysplasia-associated lesion or mass) were also excluded, as well as one patient, known to have a sessile cancer, who had declined operation and one patient who died (unrelated cause) after colonoscopic diagnosis but before intervention.

Continuous variables such as age and polyp size are presented as median (range) or mean(s.d.). The Mann–Whitney *U* test was used to compare non-parametric data and the Student's *t* test for parametric data. Categorical data such as polyp location, resection technique and outcome were analysed with Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results

Overall 130 sessile polyps were identified in 129 patients. The initial colonoscopy was performed by one of the two specialist colonoscopists in 99 patients (56 men; median age 66 (range 25–84) years; 100 polyps) and by one of 14 non-specialists (four surgeons, three physicians and seven experienced trainees) in 30 patients (11 men; median age 72 (28–85) years; 30 polyps). Overall, polyps in the non-specialist group were larger than those in the specialist group (*Table 1*), but there was no difference in anatomical location or distribution of histological type between the two groups. For polyps that were initially managed by endoscopic polypectomy, there was no difference in the median (range) size between the specialist and non-specialist groups (25 (20–80) *versus* 30 (20–55) mm; $P = 0.20$).

Methods of initial resection for polyps that proved to be benign are detailed in *Table 2*. Specialists attempted endoscopic resection significantly more often (80 of 86

Table 1 Distribution, size and pathological findings for all large sessile polyps diagnosed by specialists and non-specialists

	Specialists (n = 100)	Non-specialists (n = 30)
No. distal to splenic flexure	58	24‡
Median (range) size (mm)	25 (20–80)	35 (20–80)§
Carcinoma*	14 (T1, 13; T2, 1)	10 (T1, 8; T3, 1; unknown, 1)
Adenoma†		
Tubular	26 (12, 7, 7)	4 (3, 0, 1)
Tubulovillous	39 (14, 14, 9)	8 (3, 2, 3)
Villous	15 (4, 7, 4)	5 (1, 2, 2)
Serrated	5 (4, 1, 1)	1 (0, 1, 0)
Metaplastic	1	1
Unknown	2	1

*Values in parentheses denote tumour stage. †Numbers with mild, moderate and severe dysplasia respectively shown in parentheses. ‡$P = 0.03$ *versus* specialist group (Fisher's exact test); §$P = 0.01$ (Mann–Whitney *U* test)

Table 2 Methods of initial resection for benign polyps by specialists and non-specialists

	Specialists (n = 86)		Non-specialists (n = 20)	
	n	Size (mm)*	n	Size (mm)*
Endoscopic				
No saline				
Single snare	2	20 (20–20)	3	20 (20–20)
Piecemeal	39	25 (20–80)	9	38 (20–55)
Saline injection				
Single snare	1	20 (20–20)	0	—
Piecemeal	37	30 (20–50)	2	30 (30–30)
Fulguration alone	1	25	0	—
Other (unknown technique)	0	—	1	—‡
Non-endoscopic				
Open surgery	6	35 (25–50)	4	55 (35–80)
Transanal resection	0	—	1	70

*Values are median (range). ‡Polyp size not stated but known to be greater than 2 cm cases for specialists *versus* 15 of 20 for non-specialists; $P = 0{\cdot}03$) and used saline-assisted polypectomy techniques, with either piecemeal or single-snare resection, significantly more frequently (38 of 80 cases for specialists *versus* two of 15 for non-specialists; $P = 0{\cdot}02$).

The outcomes of endoscopic management for benign polyps are detailed in *Table 3*. Complete endoscopic success was defined as occurring when no polyp recurrence was detectable at follow-up. This was achieved more frequently by specialists than by non-specialists ($P = 0{\cdot}01$). There was no significant difference, however, in the median (range) number of therapeutic procedures required to complete polypectomy by specialists and non-specialists (1 (1–4) *versus* 2 (1–2) procedures; $P = 0{\cdot}2$). No polyp in either group, once declared completely resected using the above definition, has subsequently recurred. The median number of follow-up examinations after complete resection in these patients was 1 (range 1–4), with a median recurrence-free follow-up time of 11·7 (range 2·1–64·2) months. Additionally seven patients are continuing with endoscopic management although they have not had a normal follow-up examination to date.

Altogether 21 patients with benign polyps were referred for operation, either for initial resection or following failure of endoscopic polypectomy. Significant complications occurred in six of these 21 patients, including one death from respiratory failure 48 h after a right hemicolectomy, two episodes of postoperative haemorrhage (both following transanal resection), pneumonia in two patients and anastomotic dehiscence in one.

For sessile polyps that proved to be cancer, specialists attempted resection less often than non-specialists (five of 14 *versus* eight of ten cases respectively; $P = 0{\cdot}05$). There was no significant difference in size between cancers in patients who were referred directly for operation and those for which endoscopic resection was attempted. Endoscopic resection was initially attempted in 13 of 24 sessile cancers overall, two of which (a stage T2 sigmoid cancer and a stage T3 transverse colonic cancer) were advanced. The patients with advanced lesions (one each from the specialist and non-specialist groups) underwent surgery and remain disease-free at 5 and 1 years respectively. Ultimately, however, all 24 patients with cancer were referred for operation. No residual cancer was present in the surgical specimen in two patients (both polypectomies performed by a specialist endoscopist).

Complications of endoscopic resection requiring hospital admission occurred in three of the patients managed by specialists: one with delayed haemorrhage requiring transfusion but no other intervention; one with an intraprocedural bleeding episode that settled spontaneously; one with abdominal pain, but no peritonism or free gas on plain radiography, which resolved after 24 h of inpatient observation. One patient in the non-specialist group was admitted with self-limiting bleeding. There were no perforations.

The approximate cost of management for benign polyps was calculated for patients who reached the endpoint of successful endoscopic or surgical resection. Using local charges for colonoscopy (£469), operating theatre time (£205 per h) and daily inpatient rates (£300), the approximate cost of patient management was calculated. The mean cost of surgical management for benign polyps, calculated from 16 of 21 patients with available data on hospital stay (median 8·5 (range 2–45) days) and operating time (median 1·9 (range 0·4–5·6) h) was £5260. Overall management costs were considerably lower for patients managed by the specialist colonoscopists (*Table 4*). These data were not analysed statistically because the values are derived from the mean costs of surgery for benign polyps in the whole group and are therefore estimates.

Discussion

The efficacy of endoscopic resection for large sessile colonic polyps has been reported in several case series[5–7]. However,

Table 3 Outcome of endoscopic management for benign polyps by specialists and non-specialists

	Specialists ($n = 80$)		Non-specialists ($n = 15$)	
	n	Size (mm)*	n	Size (mm)*
Complete endoscopic success	61	25 (20–80)	6‡	40 (20–45)
Continuing endoscopic management	6	33 (20–50)	1	—
Failed endoscopic management	5	30 (20–50)	5	23 (20–55)
Lost to follow-up†	8	23 (20–50)	3	33 (30–35)

*Values are median (range). †Includes one patient who died from an unrelated illness during follow-up. ‡$P = 0{\cdot}01$ *versus* specialist group (Fisher's exact test)

Table 4 Cost of management for patients with benign polyps and successful endoscopic resection or surgery

	Specialists		Non-specialists	
	n	Cost (£)	*n*	Cost (£)
Total colonoscopies for successful polypectomy (cost at £469 each)	91	42 679	12	5 628
Surgery for benign polyps (cost at £5260 each)*	11	57 860	10	52 600
Total colonoscopies before surgery (cost at £469 each)	16	7 504	13	6 097
Total no. of patients in the analysis (sum cost)	72	108 043	16	64 325
Mean cost per patient		1 500		4 020

*Cost of surgery includes operation plus inpatient stay (mean for 16 of 21 patients for whom information was available)

in many UK centres patients with these polyps are still being referred directly for operation. Reluctance to attempt endoscopic removal may reflect a lack of local expertise or concern at delaying definitive treatment of a potentially malignant lesion, but exposes patients to the risks of surgical morbidity and mortality.

This study corroborates previous reports that complete and uncomplicated polypectomy can be achieved in the majority of patients. Surgery was avoided overall for 70–93 per cent of patients with benign polyps, the latter figure presupposing success in patients who were lost to follow-up or are continuing with endoscopic management. In this study successful endoscopic resection was defined as the absence of recurrence at follow-up. However, the median number of normal follow-up examinations after successful polypectomy was only 1. Most of these patients continue under surveillance and it remains to be seen whether there will be any delayed recurrences, as has been observed by others[6]. The specialists in this study, however, routinely tattooed polypectomy sites (sterile India ink) and used dye-spray (0·1 per cent indigocarmine) at subsequent examinations to facilitate accurate endoscopic follow-up.

Two of the endoscopists in this series are recognized specialist colonoscopists, each performing more than 800 colonoscopies annually. The apparent effectiveness of the specialists may have been influenced by referral bias, as tertiary referrals comprised 30 per cent of the cases they performed. Tertiary referrals could have caused bias in either direction, depending on the referring clinician's threshold for referring difficult cases for endoscopic or surgical management. Polyps in patients referred from other centres had usually been biopsied at the time of diagnosis and were therefore more likely to be benign, explaining the smaller proportion of malignant polyps managed by the specialists.

The benign polyps in the non-specialist group were larger, probably contributing to the higher rate of operation without attempting endoscopic removal. However, there was no difference in median size between the two groups for benign polyps where endoscopic polypectomy was attempted. Thus the better outcome for these polyps in the specialist group is probably a function of expertise rather than polyp dimension.

Although it is necessary to interpret retrospective analysis with caution, the demonstration of significantly better outcomes for benign polyps managed by specialists is plausible. The specialists are adept at performing the insertion of the colonoscope efficiently and with a straight instrument, allowing more time and optimal positioning for polypectomy. They are also skilled at piecemeal and saline injection techniques, the latter being used almost exclusively by the specialists in this study.

Management of large benign sessile polyps appears in this study to have been more cost-effective in specialist hands, with considerable savings because of proportionately fewer patients being referred for operation. The prolonged hospital stay of the six surgical patients with complications contributed significantly to the difference in cost. Several authors have described laparoscopic segmental colectomy for benign polyps[8,9], which was not performed in this series. While laparoscopy accelerates recovery and shortens hospital stay, the procedure is more time consuming and therefore more costly. Greater cost-effectiveness of specialist care[10], and improved outcomes with higher case volume per individual surgeon[11] or hospital[12], have also been demonstrated in other disciplines.

New endoscopic techniques that allow large *en bloc* mucosal resections[7,13–15], and even full-thickness endoscopic colotomy[16], are likely further to improve the likelihood of achieving complete endoscopic polypectomy, although the level of skill required to perfect these techniques will confine their use to specialist centres.

Sessile polyps that have undergone malignant transformation may have atypical features, including surface ulceration and irregularity, or firmness to palpation[17,18]. It is known that invasion beyond the first third of the submucosa is associated with a significant risk of lymph node invasion[19], but accurate pathological staging of specimens resected by piecemeal polypectomy is difficult because of fragmentation and artefact. Therefore it is important to identify invasive disease early, to avoid unnecessary and possibly dangerous attempts at endoscopic resection, and to enable expeditious surgical management. In this study the specialist colonoscopists were significantly better than non-specialists at recognizing the features of malignancy in polyps and so were less likely to attempt endoscopic resection. Their greater accuracy presumably derives from familiarity with the features of malignancy, as well as the use of saline injection techniques to elicit the 'non-lift sign'[17], which usually signifies invasive disease. Even the specialists, however, failed to recognize one-third of the cancers correctly, highlighting the need for more accurate techniques for *in situ* diagnosis and staging. Endoscopic ultrasonography may soon fulfil this role[20].

In conclusion, the majority of large benign sessile polyps in the colon can be safely and successfully removed endoscopically. For polyps that have been judged to be endoscopically irresectable, the opinion of a specialist colonoscopist should be sought before considering operation. Management by a colonoscopy specialist not only improves outcome but is also more cost-effective.

Acknowledgements

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Short note

Per-rectal drainage of anastomotic abscess in patients with rectal cancer who have received preoperative radiotherapy

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Introduction

Perianastomotic abscess formation can complicate surgery for rectal cancer. Patients who have received preoperative radiotherapy experience a higher than expected anastomotic leak rate (8–15 per cent)[1,2] and problems remain with healing of ultra-low anastomoses[3]. Patients may present with pain in the lower abdomen, peranal discharge and/or pyrexia. The presence of an anastomotic dehiscence and leakage into the perirectal cavity is readily recognized by water-soluble contrast proctography. Pelvic ultrasonography or computed tomography (CT) may demonstrate an abscess cavity but fail to demonstrate the site of dehiscence satisfactorily. Surgical drainage, ultrasonographically guided transperineal drainage or CT-guided transgluteal drainage may achieve complete resolution of the abscess cavity and closure of the fistulous communication between the abscess cavity and the residual rectum. This paper describes a simple technique to achieve drainage by the per-rectal route under fluoroscopic control.

Patients and methods

Over a 6-year period from 1992 to 1998, 152 patients had rectal cancer surgery with a primary anastomosis. Some 57 patients had preoperative radiotherapy, of whom eight developed a perianastomotic abscess. Of the eight patients, six had a defunctioning ileostomy. The mean age of the patients was 69 (range 51–82) years. There were seven men and one woman. The radiation dose received was 20 cGy in seven patients and 50 cGy in one. The mean time from operation to detection of the abscess was 16 (range 8–39) days. CT was performed in two patients and water-soluble contrast proctography in all eight. Under fluoroscopic guidance a 0·035-inch (0·89 mm) stiff guidewire was passed into the rectum. An 8-Fr guiding catheter with a small curve at the end was passed over the wire to enable manipulation of the wire into the abscess cavity across the dehiscence. Occasionally a glide wire (Terumo®; Terumo Corporation, Tokyo, Japan) was used to enter the cavity. Over the wire the guiding catheter was exchanged for a locking pigtail drainage catheter (8–14 Fr) (*Fig. 1*). The drainage catheter was fixed to the medial aspect of the thigh using an adhesive plaster and attached to a suction drainage bag. In one patient with a wide anastomotic defect and a large cavity, a Foley catheter was placed in the cavity instead of the pigtail catheter. Serial proctography was performed at unspecified intervals until there was no drainage. Dislodgement of the catheter was managed by repositioning.

Results

Seventeen drainage procedures were performed in eight patients, with a mean of 2·1 (range 1–4) procedures per patient. The mean period of drainage was 49 (range 4–152) days. Complete resolution of the abscesses and closure of the fistulous communication was achieved in seven of the eight patients. This was confirmed by a normal water-soluble contrast proctogram. The remaining patient was managed by abdominoperineal resection. The failure to

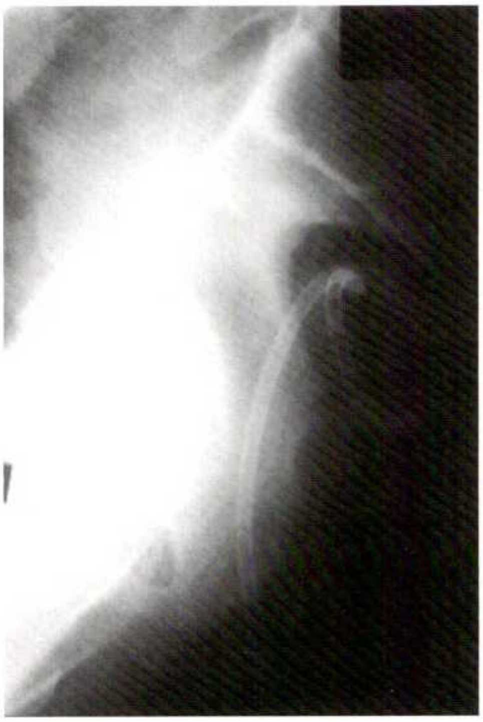

Fig. 1 Lateral view of presacral space with pigtail catheter positioned for drainage of anastomotic abscess

heal was attributed to a wide perianastomotic communication with the abscess cavity.

Discussion

Perianastomotic abscess formation is a potential complication of low anterior resection with or without loop ileostomy. Preoperative radiotherapy may or may not increase the incidence of this complication. Higher radiation doses have resulted in poor healing and increased occurrence of anastomotic dehiscence[1]. Water-soluble proctography demonstrates the site and extent of the anastomotic dehiscence and the perianastomotic abscess cavity. CT may be useful to define the relation of the cavity to the anastomotic site, but in two of the present patients it did not demonstrate the site of dehiscence with good confidence. Evaluation of a larger group may be necessary to define the role of CT in the evaluation of this complication. In the authors' opinion, water-soluble proctography is a more dynamic investigation to demonstrate this complication, and drainage of the abscess cavity may be carried out at the same time.

This study demonstrates healing of perianastomotic abscess collections when drained by the per-rectal route, although in most cases there was a long time to resolution. Usually an attempt at conservative therapy is made in all patients, reserving surgical reintervention for repair or drainage in patients whose anastomotic dehiscence fails to close spontaneously. Fluoroscopically guided per-rectal drainage of perianastomotic abscess is easily achieved with satisfactory closure of anastomotic dehiscence. Compared with CT-guided transgluteal drainage or ultrasonographically guided transperoneal drainage methods, fluoroscopically guided per-rectal drainage is a painless procedure, easily repeatable for repositioning, and requires no special preparation or anaesthesia. Disadvantages include patient discomfort from the catheter and the potential need for repositioning of the catheter. Long drainage times are to be anticipated, with a mean of 49 days seen in the present study. The prolonged drainage forms a mature fistulous tract at the dehiscence. In most patients this tract will heal after removal of the catheter[4]. Several methods of per-rectal drain insertion have been described[5,6], and may be modified to the operator's convenience and patient suitability.

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Original article

Anorectal functional disorders in the absence of anorectal inflammation in patients with Crohn's disease

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Background: Histological alterations in the enteric nervous system (ENS) have been described in patients suffering from Crohn's disease (CD). The aim of this study was to investigate whether patients with CD without rectal inflammation have abnormal anorectal function compared with healthy volunteers.
Methods: Fifty-four patients with CD and 26 healthy volunteers were examined by anorectal manometry and answered a standardized questionnaire. No patient had active CD in the rectum as determined by endoscopy.
Results: Maximum anal resting and squeeze pressures did not differ between patients and healthy volunteers. The rectoanal inhibitory reflex was absent in 24 of 54 patients and two of 26 healthy volunteers ($P < 0{\cdot}05$). The first sensation to distension of the rectal balloon was reported at mean(s.e.m.) 57·9(4·4) ml by patients and 37·5(2·2) ml by healthy volunteers ($P < 0{\cdot}01$). The standardized interview revealed additional disorders of anorectal function in patients with CD.
Conclusion: Anorectal function appears to be altered in many patients with CD even in the absence of macroscopic anorectal disease. This may be due to a disorder of the ENS.

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Introduction

The aetiology of Crohn's disease (CD) is unknown and knowledge of the pathophysiology of CD is very limited[1]. Immunological, microbiological and neurohumoral mechanisms have been suggested[2–4], and the role of the enteric nervous system (ENS) has been studied[5,6]. Alterations in neuropeptide content of the enteric neurones have been identified in CD by immunohistochemistry[7–9]. Furthermore, morphological changes of enteric neurones have been observed by electron microscopy even in macroscopically or histologically normal bowel[10,11]. Changes of neurotransmitter coding in neurones of the submucosus plexus in non-inflamed rectum of patients with CD have been demonstrated recently[12].

The significance of these observations is not clear. It is possible that a generalized neuropathy is present in CD which may also involve the ENS[13,14]. Evidence exists from animal studies that, in the intestine, interaction between nerve fibres and immune cells can occur that may be relevant to the regulation of local inflammatory responses to pathogenic materials that enter the organism via the oral route[15,16]. The aetiology of CD may therefore include not only a dysfunction of the gut immune system but also a disorder in intestinal innervation.

Although histological changes are observed in the ENS of patients with CD even in the absence of local inflammation, it is unknown whether this is accompanied by a functional disorder of the ENS. The function of the ENS is difficult to investigate directly in the small intestine and the colon. However, the rectoanal inhibitory reflex (RAIR) is a simple test for the functional integrity of the ENS in the anorectum[17–19].

The aim of present study was to investigate whether anorectal functional disorders are present in patients with CD who have macroscopically uninvolved anorectum.

Patients and methods

Patients

Fifty-four patients with CD, 30 women and 24 men of median age 37 (range 18–88) years, were studied. Forty-six patients had quiescent or minimally active CD (CD activity index (CDAI) less than 150); eight patients had a CDAI greater than 150. CD affected the colon only in three patients, the small bowel in three, and both the colon and small bowel in 48 patients. Thirty-three patients had undergone abdominal surgery in the past. Five patients had previously had a single episode of perianal sepsis that required subcutaneous abscess drainage without involvement of the anal sphincters and that was completely healed by the time of investigation. No patient had previously had extensive anorectal surgery, perianal fistula, anal fissure, diabetes mellitus, anorectal malformation or active rectal CD. All patients were receiving maintenance or therapeutic medication for CD (corticosteroids $n = 20$, sulphasalazine $n = 15$, metronidazole $n = 2$, azathioprine $n = 1$, loperamide or cholestyramine $n = 14$). Furthermore, 26 healthy volunteers, 16 women and ten men of median age 28 (range 21–47) years, with normal anorectal function and no previous abdominal surgery acted as controls.

Standardized interview

To establish the degree of anorectal continence before anorectal manometry, patients were interviewed with respect to the number of bowel movements per day; predominant consistency of stool when trying to defaecate; continence to gas, liquid and solid stool; ability to distinguish stool consistency before defaecation; maximum time to postpone defaecation; and use of sanitary pads.

Anorectal manometry

Anorectal manometry was performed in the right lateral position. An eight-channel, water-perfused manometry system (Arndorfer Medical Specialties, Greendale, Wisconsin, USA) was used for pressure recordings. The recording points were located at the tip of a catheter (length 90 cm, outside diameter 4·8 mm) at a distance of 0·5 cm in a 45° radial position (ARM[3] 8 Standard Anorectal Catheter; Arndorfer Medical Specialties). A ninth lumen was used for air insufflation into a latex balloon 3·5 cm beyond the distal recording point and for pressure measurement in this balloon.

Pressures in the anal canal were transmitted to pressure transducers (Statham transducer, Model P23XL; Spectramed, Oxnard, California, USA) and relayed to a personal computer via an analogue to digital converter (Combi-interface, PC-Polygraf VIII; Synetics Medical, Frankfurt, Germany). Data analysis was performed with dedicated software under visual control on the computer monitor (Polygram Software, Version 4.21; Synetics Medical).

Maximum anal resting pressure was measured by a continuous pull-through (speed 0·1 cm/s). Maximum squeeze pressure was determined as the maximum pressure above baseline by a stationary pull-through technique according to Coller[20]. After positioning the outer channel above the external sphincter, the catheter was withdrawn in 1-cm increments. After each withdrawal the subject was asked to squeeze for approximately 10 s after a rest of 20 s. To determine the RAIR volumes of 10, 20, 30, 40 and 50 ml of air were successively insufflated into the anorectal balloon at a speed of 10 ml/s. Thresholds for each patient's minimal perception and urgency to defaecate were investigated by rectal balloon distension. Rectal compliance was assessed at maximum tolerable volume, calculated as the volume per balloon pressure in the rectum minus balloon pressure outside the rectum.

Data analysis

Descriptive data are given as mean(s.e.m.). Differences between healthy volunteers and patients with CD were assessed with the Mann–Whitney rank sum test. Proportions were compared using the χ^2 test. $P < 0.05$ was considered statistically significant.

Results

Anorectal manometry

The RAIR was absent in 24 of the 54 patients with CD and in two of the 26 normal volunteers ($P < 0.05$). Maximum anal resting pressure was 82·2(3·6) mmHg in patients with CD and 90·2(5·3) mmHg in healthy volunteers (P not significant). Maximum squeeze pressures were 124·7(6·6) and 147·9(10·9) mmHg respectively (P not significant). The first sensation during intrarectal balloon distension occurred at 37·5(2·2) ml in healthy volunteers and 57·9(4·4) ml in patients with CD ($P < 0.01$), while the balloon volume at the onset of the urge to defaecate was 183·9(16·8) compared with 160·2(10·8) ml (P not significant). Rectal compliance at maximum tolerable volume did not differ between healthy volunteers and patients with CD (6·5(0·5) *versus* 5·1(0·3) ml/mmHg).

Standardized interview

The frequency of bowel movements was 4·5(0·8) per day in patients with CD compared with 1·9(0·2) in healthy volunteers ($P < 0.05$). All healthy volunteers were fully continent, while 24 per cent of the patients with CD had incontinence to gas (P not significant), 46 per cent to liquid stool ($P < 0.05$) and 9 per cent to solid stool (P not significant). Half of the patients with CD and all normal volunteers were able to delay a bowel movement for more than 5 min ($P < 0.05$). Thirty-nine of the 54 patients with

CD and five of the 26 normal volunteers had liquid stools ($P < 0\cdot05$). All 26 healthy volunteers were able to discriminate between different stool consistencies before defaecation but only 41 (76 per cent) of the 54 patients with CD (*P* not significant); and 12 (23 per cent) of the 54 patients but no healthy volunteer used sanitary pads (*P* not significant) (*Table 1*).

Discussion

In the present study, anorectal function of patients with CD without obvious disease of the anorectum was investigated and compared with that in healthy volunteers. The ENS-dependent RAIR was absent up to a rectal distension of 50 ml in 24 of the 54 patients with CD and two of the 26 volunteers who served as controls. No differences in anal sphincter pressure, rectal compliance or maximum tolerable volume at distension were observed. However, in patients with CD the sensory threshold to distension of a rectal balloon was present at higher volumes. Patients with CD also had more frequent bowel movements, a higher rate of faecal incontinence, and more frequent difficulties in discrimination of stool consistency before defaecation.

The RAIR, which has been described as a sampling reflex[21–23], is triggered by mild distension of the lower rectum by stool or gas. It consists of a reflex relaxation of the internal anal sphincter[17,24] that allows rectal contents to reach the highly sensitive transition zone of the anal canal. Thus, differentiation between gas, liquids or solid stool is possible, while defaecation can still be delayed if contraction of the external anal sphincter[18,25] moves the bolus back into the rectum. Discrimination of rectal contents becomes difficult if the RAIR is absent[22,26].

The RAIR has been described as a reflex within the rectal wall that is independent of the extrinsic innervation to the rectum[17,24]. Furthermore, the integrity of the ENS is indispensible for this reflex as it is absent in aganglionosis coli (Hirschsprung's disease) or after transection of the lower rectum following low anterior rectal resection[19,27,28]. Thus, the presence of the RAIR may serve as a simple test for the functional integrity of the ENS in the lower rectum.

Although the RAIR could not be elicited by distension of the rectum up to 50 ml in 44 per cent of the present patients with CD, it may have been elicited at higher distension volumes. Nevertheless, in comparison to the normal volunteers, it is clear that the RAIR in patients with CD was at least severely blunted. Thus, dysfunction of the RAIR was present in almost half of the patients with CD investigated. Dysfunction of the RAIR in many patients with CD without apparent alteration in external sphincter function (maximum squeeze and maximum resting pressure) suggests dysfunction of the ENS. These results are not consistent with a more generalized neuropathy of the peripheral nervous system, because the extrinsic innervation to the anal sphincters was apparently unaffected. This dysfunction may be limited to the rectum and lower anal canal. However, other studies have described generalized morphological alterations in the ENS in patients with CD in macroscopically non-inflamed areas. Dvorak *et al.*[10,11,29] described severe and extensive submucosal axonal damage and necrosis in patients with CD. Identical axonal necrosis was identified both in affected and apparently normal areas.

Table 1 Data from standardized interview

	Patients with CD ($n = 54$)	Healthy volunteers ($n = 26$)
Bowel movements (per day)*	4·5(0·8)‡	1·9(0·2)
Consistency of stool†		
Gas	5 (9)	0 (0)
Liquid	39 (72)¶	5 (19)
Solid	10 (19)¶	21 (81)
Use of sanitary pads†	12 (23)	0 (0)
Continence†		
To gas	41 (76)	26 (100)
To liquid stool	29 (54)¶	26 (100)
To solid stool	49 (91)	26 (100)
Ability to discriminate stool consistency before defaecation†	41 (76)	26 (100)
Maximum time to postpone defaecation at urge (min)†		
< 5	27 (50)¶	0 (0)
> 5	27 (50)¶	26 (100)

*Values are mean(s.e.m.); †values in parentheses are percentages. CD, Crohn's disease. ‡$P < 0\cdot05$ *versus* healthy volunteers (unpaired Student's t test); ¶$P<0\cdot05$ *versus* healthy volunteers (χ^2 test)

In a previous study the present authors compared neurotransmitter coding in non-inflamed rectum of patients with CD and healthy controls[12]. Changes of neurotransmitter coding were found in neurones of the submucosus plexus of patients with CD, and a distinct population of neurones expressing vasoactive intestinal peptide was found in patients with CD but not in controls. Furthermore, a thickening of calcitonin gene-related peptide varicosities occurred in patients with CD. These findings suggest that changes in the ENS, and the nerves supplying it, may play a role in RAIR dysfunction in patients with CD. It is also possible that dysfunction of the ENS is present in other parts of the gut, where the function of the ENS is at present more difficult to investigate.

A disorder of the ENS may be a primary disorder leading to CD. However, it is impossible on the basis of the data presented to differentiate between a primary disorder in the ENS and secondary changes that follow intestinal inflammation. Earlier manifestations of CD in the anal canal may have already altered the ENS and it is possible that previous damage to the anal canal may have occurred. However, this appears unlikely as no patient had had a perianal fistula or major perianal surgery in the past and only a few patients had undergone subcutaneous abscess drainage which does not affect anorectal function[30,31]. Since drugs such as steroids or azathioprine, which are commonly used to treat CD, are not known to affect anorectal function, their use hardly explains the absence of the RAIR in so many patients with CD. Finally, inflammation in one part of the gut may cause alterations in the physiology of other distant areas of the bowel. Scott *et al.*[32] demonstrated that intestinal inflammation in the small intestine also results in activation of immune cells in the colon. Thus, manifestation of CD in one part of the gut may affect the physiology of the immune system in other gut regions that are macroscopically uninvolved. Owing to the complex interaction between immune cells and the ENS[33,34], a latent inflammation in macroscopically uninvolved gut appears feasible[10,11], and this could lead to dysfunction of the ENS.

Patients with CD needed higher rectal distension volumes to reach the level of first sensation than volunteers. A similar observation has recently been made in patients with ulcerative colitis without relevant rectal inflammation[34]. Altered viscoelastic properties are unlikely to account for this as rectal compliance was similar in patients with CD and controls. Rectal capacity was investigated in a previous study that demonstrated no difference between normal volunteers and patients with CD without rectal inflammation[35]. In contrast, increased sensitivity to distension has been described in the inflamed rectum[36].

Another striking finding of this study was the high prevalence of impaired faecal continence in patients with CD. Although faecal incontinence is a frequent problem in patients with CD[37,38], external and internal anal sphincter function was similar in patients with CD and controls. Therefore, differences in faecal continence cannot be explained by differences in sphincter pressure. A reduced rectal reservoir or reduced adaptive relaxation of the rectum for the storage of faeces may also impair faecal continence[39]. However, this was unlikely to explain differences in faecal continence between patients with CD and controls, as there were no differences in rectal compliance and the maximum tolerated volume. Because the RAIR was absent in almost half of the patients with CD, it is likely that an intact RAIR is of major importance for continence in patients with CD. An impaired RAIR may have been especially significant for these patients as they frequently experienced episodes with loose stools and had higher stool frequencies than controls.

In conclusion, dysfunction of the RAIR is common in patients with CD compared with healthy volunteers. This is indicative of a functional disorder in the ENS. It is not known whether this is a generalized disorder or merely confined to the anorectum, and its pathogenesis remains to be determined.

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Original article

Multiple sectioning and immunohistochemical staining of sentinel nodes in patients with breast cancer

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Background: The aim of the present study was to investigate whether focused analysis of sentinel nodes is more useful than routine haematoxylin and eosin examination of axillary lymph nodes obtained by axillary lymph node dissection.

Methods: One hundred and fifty-two patients with breast cancer with clinically negative axillary nodes underwent successful sentinel node biopsy using a combination of dye and radioisotope, followed immediately by standard level I and II axillary lymph node dissection. Multiple sectioning, with haematoxylin and eosin and immunohistochemical analysis of sentinel nodes using cytokeratin antibody, was compared with single section and haematoxylin and eosin analysis of sentinel and non-sentinel nodes (routine examination).

Results: A mean of 1·9 (range 1–12) sentinel nodes and 11·2 (range 4–24) non-sentinel nodes were excised per patient. Metastases were detected in 44 patients (29 per cent) by single section and haematoxylin and eosin analysis of sentinel and non-sentinel nodes. An additional five patients (3 per cent) with metastases were detected by multiple sectioning and haematoxylin and eosin analysis of sentinel nodes. A further 20 patients (13 per cent) with metastases were identified by multiple sectioning and immunohistochemical analysis of sentinel nodes. Both haematoxylin and eosin and immunohistochemical analysis of sentinel nodes missed one patient with node metastases, which led to a false-negative rate of 1 per cent.

Conclusion: Multiple sectioning and immunohistochemical staining of sentinel nodes identified 16 per cent more patients with positive axillary lymph nodes than routine haematoxylin and eosin examination.

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Introduction

Axillary lymph node dissection for breast cancer provides precise staging and local control, but has significant long-term morbidity including upper arm pain, shoulder dysfunction, numbness and lymphoedema of the arm[1,2]. Sentinel node biopsy was developed as an alternative to axillary lymph node dissection and is associated with less morbidity, but with a high accuracy in the prediction of axillary lymph node status[3,4].

Another advantage of sentinel node biopsy is that focused analysis, such as multiple sectioning and immunohistochemical staining, is easy to perform because the mean number of sentinel nodes is only one to two per patient. Some researchers have reported that conventional single-section pathological analysis of axillary lymph nodes by haematoxylin and eosin staining fails to identify prognostically significant micrometastases in many patients. In the International (Ludwig) Breast Cancer Study Group, 9 per cent of 921 patients with negative axillary lymph nodes on routine haematoxylin and eosin single-section analysis were found to be node positive on serial sectioning[5]. These women had a significantly poorer 5-year disease-free

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The Editors have satisfied themselves that all authors have contributed significantly to this publication

survival. The present study investigated whether focused analysis of sentinel nodes was more useful than conventional examination of axillary lymph nodes obtained by axillary lymph node dissection.

Patients and methods

Patients with breast cancer and clinically negative axillary nodes underwent sentinel lymph node biopsy at the Osaka Medical Centre for Cancer and Cardiovascular Diseases between December 1998 and July 2000. Patients with multiple primary tumours, non-palpable breast cancer, previous axillary surgery or pregnancy were excluded. Written consent was obtained from all patients. Sentinel node biopsy was performed using a dye and radioisotope technique as described previously[6,7].

After sentinel node biopsy, level I and II axillary lymph node dissection was performed. Sentinel nodes and axillary lymph node dissection specimens were submitted separately for pathological examination. Each sentinel node was sliced into 2-mm sections, fixed and embedded in paraffin. A single 4-μm histological section was cut from each of these 2-mm sections. Non-sentinel nodes were also fixed and embedded in paraffin, and a single 4-μm histological section was cut. All sections were stained with haematoxylin and eosin.

If a sentinel node was tumour negative on paraffin section, an additional 4-μm section was cut and subjected to immunohistochemical staining using the avidin–biotinylated peroxidase complex technique with mouse monoclonal antibody against cytokeratin (NCL-CK19; Novocastra Laboratories, Newcastle, UK).

The status of multiple sections of each sentinel node examined by haematoxylin and eosin and immunohistochemical staining using cytokeratin antibody was compared with that of one section of each sentinel and non-sentinel node examined by routine haematoxylin and eosin staining in each patient. The biggest piece of sentinel node was selected from the haematoxylin and eosin-stained slides of sentinel nodes and evaluated as one of the axillary lymph nodes.

Results

One hundred and fifty-two patients with breast cancer and clinically negative axillary nodes underwent successful sentinel node biopsy. A mean of 1·9 (range 1–12) sentinel and 11·2 (range 4–24) non-sentinel nodes were excised per patient. Metastases were detected in 44 patients (29 per cent) by single section and haematoxylin and eosin analysis of sentinel and non-sentinel nodes. Multiple sectioning and haematoxylin and eosin staining identified 48 patients (32 per cent) with metastases in the sentinel node. Of these patients, five (3 per cent) had no metastases seen on single section and haematoxylin and eosin analysis of sentinel and non-sentinel nodes. The remaining 104 patients (68 per cent) had negative sentinel nodes by multiple sectioning and haematoxylin and eosin analysis. Only one patient (2 per cent) had metastases found by single section and haematoxylin and eosin analysis of sentinel and non-sentinel nodes alone (false negative).

Immunohistochemical staining of sentinel nodes identified a further 20 patients (13 per cent) with metastases. Of these, nine had isolated tumour cells and 11 had tumour cell clusters. Twenty-five patients (16 per cent) had metastases when examined by multiple sectioning and immunohistochemical analysis of sentinel nodes, but were negative when examined by single section and haematoxylin and eosin analysis of sentinel and non-sentinel nodes. Only one patient (1 per cent) was negative on multiple sectioning and immunohistochemical analysis of sentinel nodes but positive for metastases by single section and haematoxylin and eosin analysis of sentinel and non-sentinel nodes.

Discussion

The sentinel node is the node most likely to harbour axillary lymph node metastases from breast cancer. Turner *et al.*[8] demonstrated that the probability of non-sentinel node involvement is less than 0·1 per cent if the sentinel node is tumour free by both haematoxylin and eosin staining and immunohistochemistry. Giuliano *et al.*[4] analysed patients with only one involved lymph node and at least one sentinel node. Metastases were found in 72 per cent of sentinel nodes and in only 0·4 per cent of non-sentinel nodes ($P < 0{\cdot}001$), suggesting that sentinel nodes were significantly more likely to contain metastases. Intensive examination of the sentinel node appears to be efficient for identifying metastases. Giuliano *et al.*[9] reported that sentinel node biopsy with focused histopathological analysis was more sensitive than axillary lymph node dissection with routine lymph node analysis (42 *versus* 29 per cent; $P < 0{\cdot}03$). Dowlatshahi *et al.*[10] reported that tumour metastases were found in 12 per cent of patients when sentinel nodes were sectioned at 2-mm intervals and stained with haematoxylin and eosin, compared with 58 per cent of patients when the same sentinel nodes were sectioned serially at 0·25-mm intervals and stained with cytokeratin.

In the present study, multiple sectioning and haematoxylin and eosin analysis of sentinel nodes detected an additional five patients with metastases, 3 per cent more patients with metastases than routine examination of single sections and haematoxylin and eosin analysis of sentinel and non-sentinel nodes. Multiple sectioning and immunohistochemical analysis of sentinel nodes detected a further 20 patients with metastases, 16 per cent more than the routine examination. The false-negative rate of both haematoxylin and eosin and immunohistochemical analysis of sentinel nodes was 1 per cent.

Breast cancer recurs within 5 years of surgery in 20–30 per cent of patients with histologically negative nodes[11]. Prospective randomized trials have shown that patients with negative nodes benefit from adjuvant therapy[12]. Moreover, several studies have shown that focused analysis of axillary lymph nodes can detect micrometastases missed by routine single-section analysis. Patients with axillary lymph nodes containing micrometastases have a higher disease recurrence rate and a lower overall survival rate than those with micrometastasis-free axillary lymph nodes[5,13]. The authors have reported previously that intraoperative imprint cytology of sentinel nodes could identify micrometastases overlooked by routine postoperative pathological examination[14]. Ten of 153 sentinel nodes in 101 patients were tumour positive on intraoperative imprint cytology, but negative on haematoxylin and eosin-stained paraffin sections. Micrometastasis was found in eight of these nodes by immunohistochemistry. These findings suggest that occult nodal disease was present in more than a few patients with negative nodes and was missed by conventional histological examination.

Sentinel node metastases should be divided into three categories, macrometastases, micrometastases and isolated tumour cells; macrometastases and micrometastases may be of prognostic importance, whereas isolated tumour cells may not[15]. Dowlatshahi *et al.*[10] reported that half of patients whose micrometastases were detected by serial sectioning and cytokeratin staining had isolated tumour cells and the remainder had tumour cell clusters. Similarly, in the present study micrometastases were detected in sentinel nodes from 20 patients by immunohistochemistry, nine of whom had isolated tumour cells and 11 had tumour cell clusters smaller than 2 mm. The prognostic significance of such micrometastases is unknown at present. The interpretation of the clinical significance of micrometastases, especially isolated tumour cells, is an important issue and should influence the management of patients with breast cancer and sentinel node metastases.

Focused analysis of sentinel nodes, either by multiple sectioning and/or immunohistochemical staining, can identify significantly more patients with involved axillary nodes.

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Original article

Long-term results of surgery for early gastric cancer

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Background: Gastrectomy for early gastric cancer is widely accepted as an adequate therapeutic method. Recent developments of less invasive procedures require the identification of patients who will benefit from such an approach.

Methods: A retrospective study was undertaken of 238 patients with early gastric cancer who underwent gastrectomy from 1977 to 1999. Clinicopathological data relating to survival were evaluated.

Results: Analysis of 33 node-positive patients (14 per cent) revealed a tumour diameter greater than 20 mm ($P = 0{\cdot}011$), depressed macroscopic type ($P < 0{\cdot}05$), diffuse histological type ($P < 0{\cdot}001$), poor tumour differentiation ($P < 0{\cdot}001$) and infiltration of the submucosal layer ($P < 0{\cdot}002$) as factors associated with lymph node metastasis. Multivariate analysis found diffuse histological type to be an independent risk factor. The overall 5-year survival rate was 87 per cent, and was significantly better in patients who underwent radical lymphadenectomy than in those who had regional lymph node dissection (92 *versus* 78 per cent; $P < 0{\cdot}01$). Similarly, patients younger than 65 years had a more favourable 5-year survival rate (90 per cent) than older ones (77 per cent). Multivariate analysis with the Cox proportional hazards model confirmed patient age and type of lymphadenectomy as independent prognostic factors.

Conclusion: The findings suggest that extended lymph node dissection may be beneficial for some patients with early gastric cancer, although randomized clinical trials are needed to evaluate this observation further.

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Introduction

Gastric carcinoma remains a major cause of cancer death worldwide. While morbidity and mortality rates for gastric carcinoma are decreasing steadily in many countries, the overall outcome for patients with gastric carcinoma has not changed significantly in recent decades[1–3]. The incidence of early gastric cancer varies between countries, reaching almost 40–60 per cent of cases in Japan. The prevalence of non-advanced tumours in the United States or Europe is considerably lower, and ranges between 4 and 20 per cent[2–5]. There are no detailed clinicopathological reports of patients with early gastric cancer from Eastern European countries, where the occurrence of gastric carcinoma is relatively high. In spite of the favourable 5-year survival rate of 90 per cent achieved by Japanese surgeons, slightly worse results have been reported from other centres and there is wide discrepancy in treatment strategies. Recently, local treatment for early gastric cancer by endoscopic mucosal resection (EMR) has been increasingly accepted as an adequate therapeutic method avoiding traditional surgical complications, with 5-year survival rates exceeding 85 per cent[4,6,7]. Conversely many surgeons propose a more aggressive approach with radical lymph node dissection, resulting in prolonged survival[8–10].

In the year 2000, gastric carcinoma in Poland caused 19·2 and 7·3 deaths per 100 000 for males and females respectively[11]. As in other countries, the incidence of gastric cancer in Poland is decreasing, although Poland is still amongst those countries with high incidence and mortality rates for this carcinoma. In 1977 the Polish Gastric Cancer Study was started, with a grant from the Government and the State Committee for Scientific Research. Among the aims of this multidisciplinary and multicentre study, involving eight university surgical centres, were the improvement of detection and results of surgical therapy for early gastric cancer.

The aim of the present study was to evaluate the clinicopathological pattern of early gastric carcinoma in a large series of patients from Poland and to characterize factors influencing 5-year survival, with special reference to the extent of lymph node dissection.

Patients and methods

Between 1977 and 1999, 3696 patients underwent operation for gastric cancer in a multicentre study performed by the Polish Gastric Cancer Study Group (PGCSG). Among this population 241 patients (7 per cent) were identified as having early cancer confined to the gastric mucosa or submucosa. The documentation included demographic data, clinical findings, details of the surgical procedures, histopathological parameters and follow-up data.

Macroscopic type was assessed by applying the guidelines of the Japanese Gastric Cancer Association (JGCA): elevated (I, protruded; IIa, superficial elevated), flat (IIb, superficial flat) and depressed (type IIc, superficial depressed; III, excavated)[12]. Tumours containing two or more macroscopic types were classified according to the type with the worst prognosis. World Health Organization (WHO) and Laurén classifications were used for microscopic evaluation of the tumour[13,14]. The residual tumour classification (R) of the Union Internacional Contra la Cancrum (UICC) was applied to assess the radicality of surgery performed[14]. The regional lymph nodes were numbered and classified into groups according to the JGCA[12]. Gastric resection based on the extent of lymph node dissection was classified as follows: regional gastric resection (D1) including complete removal of level 1 lymph nodes (groups 1–6); and radical gastric resection (D2) including complete removal of level 1 and 2 lymph nodes (groups 1–11). These groupings were modified slightly depending on the location of the tumour.

In each institution all resections were performed by one to three senior staff surgeons who specialized in gastrointestinal surgery and the number of procedures ranged from 15 to 75 operations per centre annually. The extent of lymphadenectomy depended on the judgement and preference of the surgeon. Tumour location and intraoperative verification of tumour-free resection margins were used to select patients for total or subtotal stomach resection. Splenectomy was performed routinely in the extended lymphadenectomy group (D2 resection) for proximal tumours. In a D1 dissection, the spleen and tail of the pancreas were not resected. No adjuvant chemotherapy or radiotherapy was used.

To identify the characteristics of early gastric carcinoma associated with nodal metastasis, patients with and without node involvement were compared with respect to the following clinicopathological factors: age, sex, family history of gastric carcinoma, single or multiple early gastric cancer[15], tumour diameter and location, macroscopic type, grade of tumour differentiation, microscopic pattern and depth of infiltration.

Follow-up involved standardized clinical examinations. All patients were asked by mail to visit the outpatient department every 6 months after operation. In some cases the date of death was obtained from the Census Registry Office.

Statistical analysis

Univariate and multivariate analyses of risk factors associated with lymph node metastasis were performed using the χ^2 test and logistic regression models respectively. Survival data for 188 patients operated on before 1996 were analysed by means of the Kaplan–Meier method[16], and the log rank test was used for assessment of differences between prognostic factors. Multivariate analysis of factors influencing 5-year survival was performed using the Cox proportional hazards model. $P < 0{\cdot}05$ was considered statistically significant. Statistical analysis was performed with the Statistica 5·5™ (StatSoft, Tulsa, Oklahoma, USA) software package.

Results

Some 238 of the 241 patients were included in the analysis. There were 151 men (63 per cent) and 87 women (37 per cent); the mean age was 55 (range 25–87) years. Detailed clinicopathological characteristics of the study group are presented in *Table 1*. Both lymphadenectomy groups (D1 and D2) were comparable with regard to clinicopathological factors and the incidence of co-morbid conditions (especially cardiac and lung disease). Only 43 patients (18 per cent) were asymptomatic at the time of diagnosis. The most common presenting symptom, occurring in 195 patients (82 per cent), was epigastric pain or discomfort.

The most common location of early gastric cancer was the distal third of the stomach, where 51 per cent of the tumours were located (*Table 1*). Lesions within the upper and middle third of the stomach comprised 5 and 45 per cent of tumours respectively. In 15 cases (6 per cent) early gastric cancer was multifocal. Most patients (54 per cent) had a tumour diameter of less than 20 mm. Based on the Laurén classification, 138 tumours (58 per cent) were intestinal type and 100 (42 per cent) diffuse or mixed type. According to the WHO classification, nearly two-thirds of tumours (61 per cent) were well differentiated. Histopathological examination in 125 cases (53 per cent) confirmed submucosal infiltration, while in the remaining 113 (47 per cent) the tumour was limited to the gastric mucosa.

Metastases to the lymph nodes were found in 33 patients (14 per cent), and the number of positive nodes ranged from

Table 1 Patient characteristics

	Overall (n = 238)	D1 lymphadenectomy (n = 81)	D2 lymphadenectomy (n = 157)
Mean(s.d.) age (years)	55·0(12·9)	55·8(14·2)	54·8(12·4)
Sex			
M	151 (63)	50 (62)	101 (64)
F	87 (37)	31 (38)	56 (36)
Family history of gastric cancer			
Yes	15 (6)	2 (2)	13 (8)
No	223 (94)	79 (98)	144 (92)
Tumour size (mm)			
> 20	110 (46)	33 (41)	77 (49)
≤ 20	128 (54)	48 (59)	80 (51)
Location in stomach			
Upper third	11 (5)	2 (2)	9 (6)
Middle third	106 (45)	33 (41)	73 (46)
Lower third	121 (51)	46 (57)	75 (48)
Macroscopic type			
Elevated	46 (19)	17 (21)	29 (18)
Flat	38 (16)	11 (14)	27 (17)
Depressed	154 (65)	53 (65)	101 (64)
No. of lesions			
Single	223 (94)	75 (93)	148 (94)
Multiple	15 (6)	6 (7)	9 (6)
Laurén classification			
Intestinal type	138 (58)	50 (62)	88 (56)
Diffuse type	100 (42)	31 (38)	69 (44)
WHO classification			
Well differentiated	145 (61)	52 (64)	93 (59)
Poorly differentiated	93 (39)	29 (36)	64 (41)
Depth of infiltration			
Mucosa	113 (47)	36 (44)	77 (49)
Submucosa	125 (53)	45 (56)	80 (51)
Lymph node status			
Node negative	205 (86)	73 (90)	132 (84)
Node positive	33 (14)	8 (10)	25 (16)
Type of resection			
Subtotal	188 (79)	78 (96)	110 (70)*
Total	50 (21)	3 (4)	47 (30)
Mean(s.d.) no. of resected lymph nodes	12·0(8·6)	7·4(4·6)	14·5(9·2)†
Complications	35 (15)	16 (20)	19 (12)
Death	2 (1)	0 (0)	2 (1)
Mean(s.d.) length of hospital stay (days)	17·8(19·9)	16·3(18·4)	18·3(20·5)

Values in parentheses are percentages, unless stated otherwise. WHO, World Health Organization. *$P < 0{\cdot}05$ (χ^2 test); †$P < 0{\cdot}05$ (Student's t test)

1 to 13 (median 3). In five patients (2 per cent) metastases to the second station of lymph nodes were present. Positive lymph nodes were found most commonly in lymph node groups 3 and 4, according to the JGCA guidelines. No cases with skip lesions were identified and location of metastatic lymph nodes was comparable in patients who underwent D1 and D2 resections. Univariate analysis of factors predicting nodal involvement is presented in *Table 2*. Tumour diameter greater than 20 mm ($P = 0{\cdot}011$), depressed macroscopic type ($P < 0{\cdot}05$), diffuse Laurén histological type ($P < 0{\cdot}001$), poor tumour differentiation ($P < 0{\cdot}001$) and infiltration of the submucosal membrane ($P < 0{\cdot}002$) were significantly associated with positive nodes. Multivariate analysis revealed Laurén histological type to be the only independent factor associated with lymph node involvement.

A total of 188 patients (79 per cent) underwent subtotal gastric resection. Fifty patients (21 per cent) had total gastrectomy when the tumour was located in the proximal part of the stomach to obtain adequate tumour-free margins verified by intraoperative frozen sections. Splenectomy was performed in nine patients (6 per cent) for proximal tumours only in the D2 lymphadenectomy group. Regional lymphadenectomy (D1) was performed in 81

Table 2 Risk factors for lymph node metastasis by univariate analysis

	Lymph nodes		
	Non-metastatic (n = 205)	Metastatic (n = 33)	P*
Age (years)			0·10
≤ 65	142 (69)	28 (85)	
> 65	63 (31)	5 (15)	
Sex			0·08
M	132 (64)	16 (48)	
F	73 (36)	17 (52)	
Family history of gastric cancer			0·06
Yes	10 (5)	5 (15)	
No	195 (95)	28 (85)	
No. of lesions			0·27
Single	194 (95)	29 (88)	
Multiple	11 (5)	4 (12)	
Tumour location			0·64
Proximal	9 (4)	2 (6)	
Middle	94 (46)	12 (36)	
Distal	102 (50)	19 (58)	
Tumour size (mm)			0·011
≤ 20	117 (57)	11 (33)	
> 20	88 (43)	22 (67)	
Macroscopic type			0·024
Elevated	43 (21)	3 (9)	
Flat	35 (17)	3 (9)	
Depressed	127 (62)	27 (82)	
Laurén classification			< 0·001
Intestinal type	132 (64)	6 (18)	
Diffuse type	73 (36)	27 (82)	
WHO classification			< 0·001
Well differentiated	137 (67)	8 (24)	
Poorly differentiated	68 (33)	25 (76)	
Depth of infiltration			< 0·002
Mucosa	106 (52)	7 (21)	
Submucosa	99 (48)	26 (79)	

Values in parentheses are percentages. WHO, World Health Organization. *χ^2 test

Table 3 Prognostic factors of long-term survival by univariate analysis

	5-year survival (%)	P*
Age (years)		0·033
≤ 65	90	
> 65	77	
Lymphadenectomy		0·008
D1 dissection	78	
D2 dissection	92	
Tumour size (mm)		0·57
≤ 20	90	
> 20	85	
Macroscopic type		0·26
Elevated	86	
Flat	75	
Depressed	87	
Laurén classification		0·31
Intestinal type	85	
Diffuse type	90	
WHO classification		0·54
Well differentiated	85	
Poorly differentiated	89	
Depth of infiltration		0·24
Mucosa	89	
Submucosa	85	
Lymph node status		0·91
Node positive	87	
Node negative	86	
Type of resection		0·71
Subtotal	85	
Total	88	

WHO, World Health Organization. *Log rank test

patients (34 per cent), and the remaining 157 (66 per cent) had a D2 resection. The mean(s.d.) number of lymph nodes retrieved in the surgical specimen was 12·0(8·6), significantly higher for D2 (14·5(9·2) nodes) than for D1 (7·4(4·6)) lymphadenectomy (P < 0·05). All resections were assessed as R0.

The overall postoperative morbidity and mortality rates were 15 per cent (35 patients) and 1 per cent (two patients) respectively, with no significant difference between the lymphadenectomy groups. Because the number of D2 resections with splenectomy was limited, no relationship could be identified between splenectomy and postoperative complications. There were no differences in complication rates between members of the PGCSG. Cardiopulmonary complications were the most most common (20 patients). Remaining complications included wound infection (n = 11; 5 per cent), upper gastrointestinal bleeding (n = 2; 1 per cent) and anastomotic leakage with subsequent abdominal abscess (n = 2; 1 per cent). Two patients with concomitant ischaemic heart disease died in the postoperative period from myocardial infarction.

Data for survival analysis were available for all 188 patients (100 per cent) operated on before 1996; the overall 5-year survival rate was 87 per cent. Factors associated with survival determined by Kaplan–Meier and log rank analysis are presented in *Table 3*. On univariate analysis, there was no significant impact of tumour size, histopathological type or tumour differentiation. Moreover, a similar 5-year survival rate was observed regardless of the depth of infiltration (mucosa *versus* submucosa), lymph node involvement or the presence of concomitant cardiac or pulmonary disease. The only factors associated with prolonged survival were extended lymphadenectomy (92 *versus* 78 per cent; P = 0·008) (*Fig. 1* and *Table 3*) and age below 65 years (90 *versus* 77 per cent; P = 0·033) (*Table 3*). The multivariate Cox regression model showed that regional

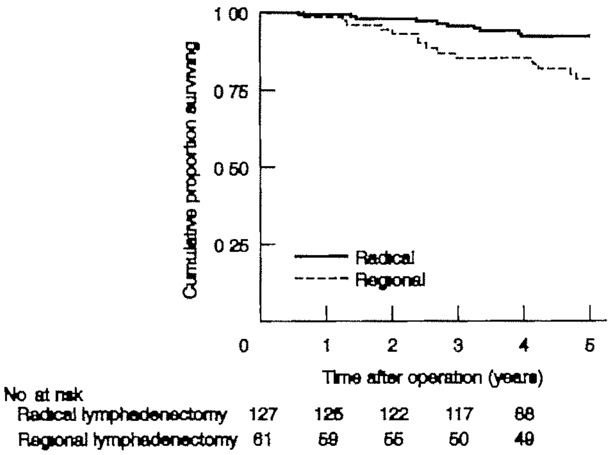

Fig. 1 Cumulative survival of patients treated by radical and regional lymphadenectomy. $P = 0{\cdot}008$ (log rank test)

lymphadenectomy ($P < 0{\cdot}014$) and age greater than 65 years ($P < 0{\cdot}035$) were independent risk factors.

Discussion

The prognosis of patients with early gastric cancer remains very favourable compared with that for advanced tumours. Unfortunately, except in Japan, the incidence of early cancer is low, ranging from 10 to 20 per cent of patients with gastric cancer[1,3–5,17–19]. In spite of encouraging 5-year survival rates for early gastric carcinoma, reported to be as high as 90 per cent, many controversial questions have been raised concerning the optimal therapeutic approach. Some authors have suggested that early gastric cancer can be treated effectively by less invasive surgery and endoscopic techniques in selected groups of patients[20–23]. Following EMR, the average survival is comparable to the results of open surgery with a recurrence rate of about 5 per cent. In contrast to open operation, EMR and laparoscopic procedures reported recently were performed almost without complications. On the other hand, other authorities advocate radical gastrectomy with *en bloc* lymphadenectomy for all patients[19,24,25].

Although the incidence of lymph node metastases in early gastric carcinoma is low, numerous studies have shown that the presence of metastatic nodes is associated with a worse prognosis[2,4,18,23,26–29]. Many authors have therefore attempted to identify clinicopathological criteria that predict a low probability of nodal involvement. The presence of nodal metastases is closely related to submucosal invasion, tumour size, poor differentiation and lymphatic invasion[21,26–30]. Numerous studies have also shown the importance of macroscopic type according to the JGCA and assessment of tumour growth pattern[27,28,30,31]. The present study indicated that diffuse type, poor differentiation, tumour diameter greater than 20 mm, infiltration of the gastric submucosa and depressed macroscopic type were all associated with lymph node involvement. Contrary to other studies, however, the presence of lymph node metastasis was not a prognostic factor in 5-year survival. Such findings have been reported rarely[32,33] and may suggest that the primary lesions were underestimated.

The average number of lymph nodes dissected in both groups is relatively low and depends largely on the diligence of the pathologist. Because low lymph node count could result in understaging, stage migration could be occurring here. The inappropriate assessment of lymph node status is probably due to the tumour emboli that are frequently found in the peripheral sinusoids of the nodes and can be easily overlooked[10,34]. Recent studies have shown the significance of such micrometastases by means of immunohistochemical techniques[34–38]. Identification of micrometastatic disease in lymph nodes, missed by routine haematoxylin and eosin staining, results in tumour upstaging and is associated with a less favourable prognosis[37,38]. Similar observations have been made regarding microinvasion of tumour cells in the muscularis propria[38].

Although the characteristics of patients with early gastric cancer and lymph node metastases have been reported from many institutions, the extent of lymph node dissection for early gastric carcinoma remains controversial. Based on a low probability of lymph node involvement, some authors recommend limited surgery or endoscopic techniques in the treatment of selected patients with early gastric cancer. Unfortunately, even with endoscopic ultrasonography, it is difficult to predict the depth of invasion and to exclude nodal metastasis confidently before operation[6,39,40]. In addition, the presence of synchronous lesions in 5–13 per cent of cases often means that total gastrectomy is required to obtain adequate resection margins[41].

Although metastatic lymph nodes are usually found to affect outcome adversely, many studies report significant benefits following radical lymphadenectomy in early gastric cancer[8,19,24,42], although others suggest only local dissection[23]. The present study revealed that radical lymphadenectomy significantly prolonged the 5-year survival rate (92 *versus* 78 per cent; $P < 0{\cdot}01$) regardless of lymph node status (*Fig. 1*), although the influence of the type of lymphadenectomy performed on survival in node-positive cases was not assessed because of the small study group size.

It is still not clear why D2 resection prolongs survival. This observation can be explained in two ways. The incidence of metastases to the second tier of lymph nodes and skip metastases to extraperigastric lymph nodes is reported in up to 9 and 7 per cent of cases respectively[4,17,19,29]. Thus, a D1 lymphadenectomy will fail to identify positive nodes at this level. Radical dissection of lymph nodes eliminates micrometastatic disease and prevents further dissemination of tumour cells.

Similar morbidity and mortality rates observed in the present study, regardless of the extent of lymphadenectomy, suggest that radical dissection does not increase the perioperative complication rate, an observation made by others[19,23,24]. Two large randomized studies have, however, demonstrated higher mortality and morbidity rates associated with extended lymphadenectomy[43–45], related largely to distal pancreatectomy and splenectomy, which are not necessary during gastrectomy for early gastric carcinoma. Resection of the spleen and tail of the pancreas may be necessary for the complete removal of lymph node stations 10 and 11 in patients with proximal tumours. As proximal tumours in the extended lymphadenectomy group occurred in only 6 per cent of cases (nine patients), splenectomy was performed only in this small group of patients. This may explain the discrepancy in the rates of postoperative complications between this study and the above-mentioned trials.

The prognosis of patients with early gastric cancer confined to the mucosa is usually reported to be better than when there is invasion of the submucosa[19,27,30]. Nevertheless, some authors have shown that depth of infiltration does not influence long-term outcome in patients with early gastric carcinoma[23,24,32,46]. The present findings revealed that the 5-year survival rate in cases limited to the gastric mucosa (89 per cent) was slightly better than that for patients with tumours infiltrating the submucosa (85 per cent), although this difference was not statistically significant. Age was an independent prognostic factor in this study, which has not been consistently described by others. There is some variation concerning the relevance of many other prognostic factors, although undifferentiated, diffuse type and larger tumours are more consistently associated with a worse long-term survival[4,17–19,24,27,30,32,46,47].

Preoperative and operative staging of early gastric carcinoma is still inaccurate, and the depth of tumour penetration or lymph node status in individual cases cannot be decided with certainty before histopathological confirmation. Furthermore, gastrectomy with extended lymphadenectomy can be performed with a low complication rate and was found to improve 5-year survival significantly.

As this study was non-randomized, there may be an element of bias. Therefore, the final conclusion that extended lymph node dissection (D2) is beneficial for patients with early gastric cancer should be considered carefully: randomized trials are needed.

Evaluation of more accurate systems of staging for early gastric cancer (i.e. growth pattern according to Kodama – classification based on tumour size, depth of infiltration and type of invasion of the muscularis mucosa) or molecular analysis of lymph node micrometastases may identify patients who can benefit from less aggressive therapy.

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Original article

Influence of implantation interval on the long-term biocompatibility of surgical mesh

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Background: The aim was to study the long-term tissue response to polypropylene mesh.
Methods: This was a retrieval study that investigated 76 polypropylene meshes with a median implantation interval of 18 (range 2–180) months. Mesh was explanted following hernia recurrence, infection or pain. The median implantation interval was 20 (range 4–180) months in the recurrence group, 30 (range 5–48) months in the pain group and 10 (range 2–56) months in the infection group ($P < 0{\cdot}05$, infection *versus* pain or recurrence). The inflammatory response was determined by immunohistochemistry of macrophages (CD68), polymorphonuclear granulocytes (CD15) and T and B lymphocytes (CD3 and CD20). The cell turnover within the interface mesh fibre–recipient tissue was measured by TUNEL for apoptosis or DNA strand breaks, Ki67 for cell proliferation and heat-shock protein (HSP) 70 for cell stress.
Results: With the exception of HSP-70, levels of all variables decreased over time. Sex, age, type of previous operation or location of the mesh did not have a significant influence.
Conclusion: Long-term incorporated polypropylene mesh in humans has a more favourable tissue response with increasing implantation interval.

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Introduction

In the 1960s the introduction of surgical mesh was a revolutionary and unorthodox step in hernia repair. Modern hernia surgery is no longer imaginable without the application of these biomaterials, leading to about one million implantations each year worldwide. The net-like alloplastic mesh is used to close the hernia defect and, with extended overlap, to reinforce the abdominal wall.

Surgical mesh is regarded as inert and biocompatible. However, knowledge about the long-term biocompatibility and tissue response of mesh in humans is still poor, although a few reports exist[1,2]. Nearly all the available data on the biological behaviour of these implants are from animal experiments[3–15].

The aim of the present study was to evaluate the biocompatibility of surgical mesh that was explanted as a result of a clinical complication such as infection, hernia recurrence or chronic pain. All mesh specimens were collected between 1995 and 2001 at different European centres of hernia surgery and sent to the Centre of Biomaterial and Implant Pathology, Aachen. The aim was to study the local tissue reaction at the interface between surgical mesh and recipient tissue, and to identify changes that occurred since implantation, similar to a previous study[16].

Materials and methods

Tissue samples

Seventy-six full explants of a standard polypropylene mesh (Marlex®, CR Bard, Murray Hill, New Jersey, USA) were collected after revision operations at various hospitals in Europe. All mesh specimens were originally implanted for abdominal wall hernia repair. The mesh had been implanted in the groin (*n* = 49, 64 per cent) or in the anterior abdominal wall (*n* = 27, 36 per cent), either in the inguinal subfascial area using the Lichtenstein method (*n* = 29, 38 per cent), in the preperitoneal submuscular space by open or laparoscopic procedures (n = 32, 42 per cent) or in front of the fascia in an onlay position (n = 15, 20 per cent). Immediately after explantation all mesh samples were fixed in 10 per cent buffered formalin at the donating centre and sent by mail to the Institute of Pathology in Aachen, together with relevant clinical data.

Morphological study

All mesh specimens were studied by light microscopy. Tissue samples were sliced into 0·3 × 1·0 cm pieces from representative lateral parts and from the centre of the mesh, and embedded in paraffin. Additionally, visibly striking areas, such as folds or node formations, were investigated. Between ten and 15 sections 4 µm thick were stained with haematoxylin and eosin, as well as with periodic acid Schiff plus diastase and Elastic van Gieson.

Immunohistochemistry

Light microscopy was complemented by immunohistochemistry, which was performed on paraffin-embedded material using the avidin–biotin complex method with diaminobenzidine as chromogen. The procedure was repeated twice for each sample at different timepoints.

Antibodies

Antibodies used in this study included polyclonal rabbit antihuman CD3 (DAKO, Hamburg, Germany) 1 : 50 as pan-marker for T lymphocytes, polyclonal rabbit antihuman CD20 (DAKO) 1 : 50 as pan-marker for B lymphocytes, monoclonal mouse antihuman CD68 (DAKO) 1 : 50 as a pan-marker for macrophages, monoclonal antihuman CD15 (Becton Dickinson, Heidelberg, Germany) 1 : 10 as a marker for polymorphonuclear granulocytes (PMNs), polyclonal rabbit anti-heat-shock protein (HSP) 70 (DAKO) 1 : 200 and monoclonal anti-HSP-70/heat-shock core protein 70 (BIOMOL, Hamburg, Germany) 1 : 200 as markers for the cell stress response, and monoclonal human Ki67 (= MIB1) (DIANOVA, Hamburg, Germany) 1 : 10 as a marker of cell proliferation.

TUNEL

TUNEL histochemistry was performed by means of an *in situ* apoptosis detection kit (Apoptag®; Oncor, Hamburg, Germany). All steps were done according to the supplier's instructions. Briefly, paraffin-embedded sections 4 µm thick were taken from each mesh specimen, fixed to slides by heating at 60°C, deparaffinized and rehydrated. After digestion with 0·02 per cent trypsin in phosphate-buffered saline (PBS) at room temperature and washing in PBS, the sections were incubated in buffer A (potassium cacodylate 200 mmol/l, Tris 0·025 mmol/l, bovine serum albumin 0·25 mg/ml, pH 6·6) for 5 min. The sections were then incubated with a labelling solution containing terminal digoxin-labelled thymidine, biotinylated-16-2′-deoxyuridine 5′-triphosphate and cobalt chloride 1·5 mmol/l in buffer A at 37°C for 1 h. The reactions were terminated by rinsing in a buffer containing sodium chloride 300 mmol/l and sodium citrate 30 mmol/l, pH 7. The sections were washed three times in PBS for 5 min. For light microscopy, the labelled DNA fragments were visualized by incubating the sections with streptavidin-conjugated alkaline phosphatase followed by reaction with medium containing fast red as chromogen. The slides were then washed, counterstained with methyl green and mounted in Permount® medium (Merck, Darmstadt, Germany).

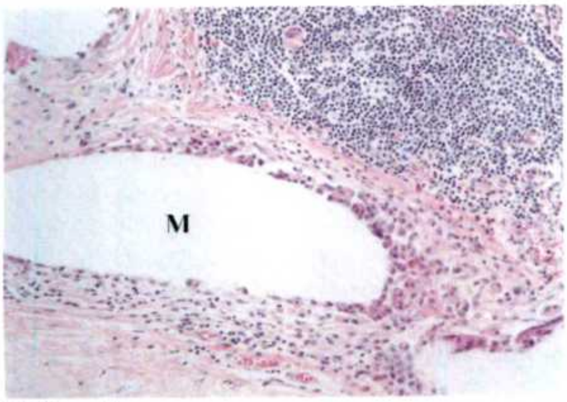
Morphometry

Morphometric evaluation consisted of a quantitative cell analysis of the inflammatory reaction and soft tissue reaction. Variables measured were the percentage share area of the inflammatory infiltrate (partial volume), connective tissue and vessels, as well as the percentage of macrophages, lymphocytes, PMNs and fibroblasts, including the expression of TUNEL, Ki67 and HSP-70 in these cells.

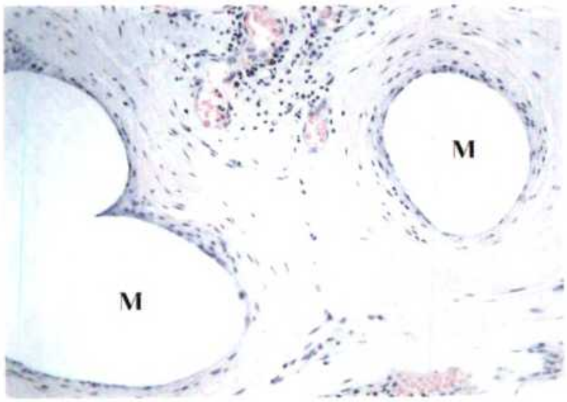
The share areas were determined in five haematoxylin and eosin-stained slides at a grid of ten fields (0–300 µm; 100 ×, area 0·1 mm^2) in ten random fields per slide. The number of cells was counted again with a grid of ten points in the interface mesh fibre–recipient tissues (0–150 µm, 400 ×, area 625 μm^2) in ten fields per slide.

Statistical analysis

The influence of the clinical variables on the tissue response was tested for significance by analysis of variance with least significant difference modification according to Bonferroni. The Pearson correlation coefficient was calculated as an indicator of the association between the implantation interval and morphometric data. Multivariate analysis was used to verify independent effects for indication, implantation time, implantation technique and mesh location, patient sex and age. Statistical significance was assumed at $P < 0.05$. All data are shown as mean(s.d.) unless otherwise stated.



a 12 months



b 180 months

Fig. 1 Typical histological aspect after **a** 12 months and **b** 180 months of implantation; note the decreased inflammatory reaction at the interface and the surrounding scar formation after 180 months (**b**). M, mesh fibre (haematoxylin and eosin stain, original magnification × 200)

Results

Between 1995 and 2001 a total of 76 explanted specimens of polypropylene mesh (Marlex®) were collected. The mesh came from 52 men and 24 women with a mean age of 55(11) and 52(15) years respectively. Eight patients were below the age of 40 years. The main indications for explantation were infection ($n = 19$, 25 per cent), chronic pain ($n = 10$, 13 per cent) or hernia recurrence ($n = 47$, 62 per cent). The median duration of incorporation was 20 (range 4–180) months in patients who had mesh removal for recurrence, 30 (range 5–48) months for pain, and 10 (range 2–56) months for infection ($P < 0\cdot05$, infection *versus* pain or recurrence). There were no significant differences between the three groups with regard to implantation procedure or patient sex. Patients with an onlay mesh were significantly older (61(13) years) than those with a preperitoneal mesh (51(12) years) ($P < 0\cdot05$) or an inguinal, subfascial mesh (54(13) years). Some 28 per cent of all reoperations for recurrent hernia were performed within 1 year of mesh implantation, 58 per cent within the first 2 years and 24 per cent after 3 years. The rate of infection was similarly distributed between both sexes and all locations.

General macroscopic and microscopic observations (*Fig. 1* and *Table 1*)

All removed polypropylene mesh had an intense scar formation with considerable shrinkage and folding. In most cases, fixation sutures were still in place. All mesh demonstrated a chronic persisting foreign body reaction with formation of a fibrous capsule, even years after implantation. Overt infection increased the local inflammatory response and in most cases decreased the induction of connective tissue.

Table 1 Tissue response around polypropylene mesh removed for hernia recurrence, pain or infection

	Recurrence	Pain	Infection
Duration of incorporation (months)	29·8(30·4)	28·3(17·0)	14·2(12·8)*
Partial volume of inflammatory infiltrate (%)	34·8(12·1)	34·6(12·4)	44·9(9·5)*
Partial volume of connective tissue (%)	41·2(8·6)	35·0(8·8)	30·8(11·7)†
Partial volume of vessels (%)	9·8(2·3)	9·6(2·4)	8·3(2·3)†
Macrophages	36·6(7·9)	35·5(5·8)	44·5(11·6)*
Polymorphonuclear granulocytes	7·8(3·1)	7·4(4·8)	18·0(3·6)*
Lymphocytes	7·0(3·2)	7·0(2·5)	9·0(3·8)†
Fibroblasts	16·7(6·5)	16·6(7·4)	13·0(5·3)†
TUNEL (% positive)	24·6(12·4)	24·5(9·1)	40·6(15·6)*
Ki67	21·1(9·5)	23·2(11·2)	31·5(13·9)†
HSP-70	50·6(21·7)	45·5(21·9)	23·7(14·1)*

Values are mean(s.d.). HSP, heat-shock protein. *$P < 0\cdot05$ *versus* recurrence or pain; †$P < 0\cdot05$ *versus* recurrence (least significant difference Bonferroni test)

A predominant foreign body reaction with typical foreign body granulomas including epitheloid cells and giant cells was observed. Smaller numbers of PMNs were detected regularly at the interface between mesh and recipient tissues. The inflammatory process was accompanied by a pronounced perifilamentous fibrosis with extensive deposition of collagen fibres. In the interface of the mesh the collagen fibres were basically orientated parallel to the polypropylene threads. In addition, the pores were filled with collagen-rich connective tissue. The polypropylene mesh was integrated in a complete three-dimensional scar plate.

Tissue and cellular response (*Fig. 2*)

There were no significant differences between mesh removed for pain and mesh removed for recurrence with regard to inflammatory partial volume, partial volume of vessels and connective tissue, and the number of macrophages, PMNs, lymphocytes, fibroblasts and cells positive for TUNEL, Ki67 or HSP-70.

Comparison of all data indicated a significant effect of incorporation interval ($P < 0{\cdot}01$), with a decrease in the partial volume of inflammatory cells ($r = -0{\cdot}651$). In contrast, the amount of connective tissue ($r = 0{\cdot}189$) and the partial volume of vessels remained constant over time ($r = 0{\cdot}047$). The amount of fat tissue correlated inversely with the level of scar formation ($r = -0{\cdot}556$).

The time course of the inflammatory infiltrate corresponded to a similar decrease in the relative cell numbers in the interface between surgical mesh and recipient tissues, in particular of macrophages ($r = -0{\cdot}478$) and PMNs ($r = -0{\cdot}522$). Simultaneously, the local cell turnover was markedly lowered (Ki67; $r = -0{\cdot}494$). The number of TUNEL-positive cells in the interface decreased with time ($r = -0{\cdot}508$, $P < 0{\cdot}01$), although in the case of mesh infection, at a raised level ($P < 0{\cdot}01$). Over time the number of fibroblasts remained almost constant or even decreased ($r = -0{\cdot}161$; $P = 0{\cdot}16$). In contrast, a marked increase in HSP-70-positive cells was detected ($r = 0{\cdot}530$) with increasing implantation interval.

A high partial volume of inflammatory infiltrate ($r = 0{\cdot}592$, $P < 0{\cdot}01$) with increased numbers of macrophages ($r = 0{\cdot}389$, $P < 0{\cdot}01$) and PMNs ($r = 0{\cdot}701$, $P < 0{\cdot}01$) was significantly associated with an increased level of TUNEL-positive and proliferating cells ($r = 0{\cdot}619$, $P < 0{\cdot}01$) and, in contrast, to a decreased expression of HSP-70 ($r = -0{\cdot}812$, $P < 0{\cdot}01$) as a marker of the cell-stress response.

Finally, the presence of infection led to a significant increase in macrophages, PMNs and lymphocytes and a decrease in fibroblasts ($P < 0{\cdot}05$).

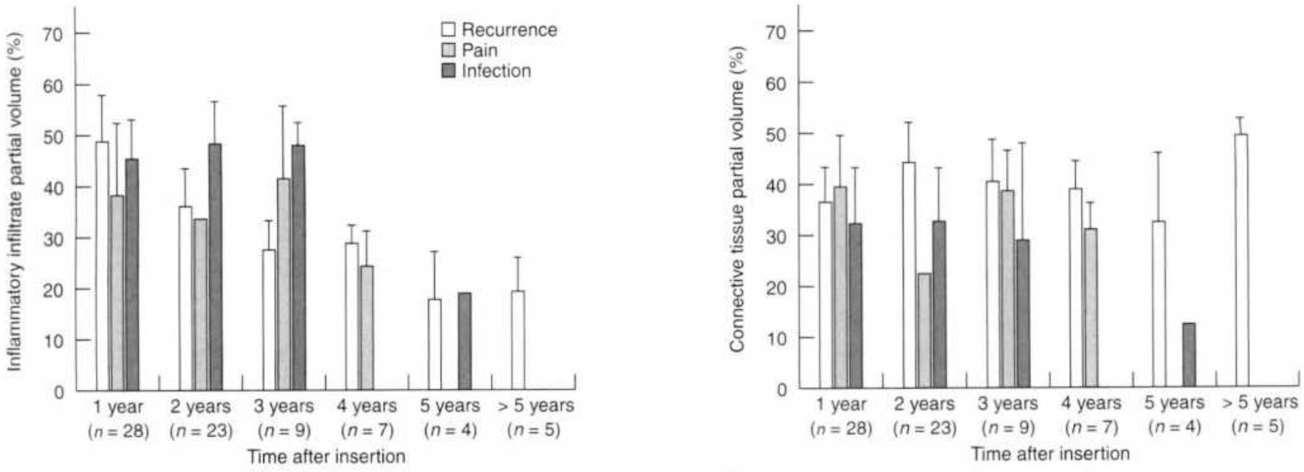
Discussion

Modern surgical hernia repair depends increasingly on synthetic mesh for reconstruction of the abdominal wall. Despite the undisputed advantage of the polypropylene mesh currently available, reports of complications after implantation are increasing. Minor local complaints such as seroma, discomfort and decreased abdominal wall mobility are frequent, and observed in about one half of patients. Serious complications such as recurrence, chronic and persisting pain and infection, including fistula formation, are rare[17–20], but sometimes force a surgeon to remove the surgical mesh. Mesh specimens obtained as a result of reoperation offer a unique insight into the long-term behaviour of these implants and therefore an overview of their biocompatibility.

It is surprising that data about the long-term biocompatibility of surgical mesh, which has now been in clinical use for more than 40 years, are not available. Mesh now represents numerically the largest group of alloplastic implants most frequently used in modern medicine. Since 1995 the authors have systematically collected and analysed explanted surgical mesh specimens. By 2001 approximately 350 samples of various mesh modifications had been gathered from hernia centres from all over Europe. In the present study a group of 76 Marlex® mesh specimens was selected to investigate the local reaction of tissues in contact with the mesh. The main questions to be answered were whether the tissue response changed over time and whether morphological data could be correlated with the reason for mesh removal. It should be stressed that the findings cannot simply be extrapolated to mesh in uncomplicated hernias; failure of mesh repair due to technical problems during the operation cannot be excluded in this study.

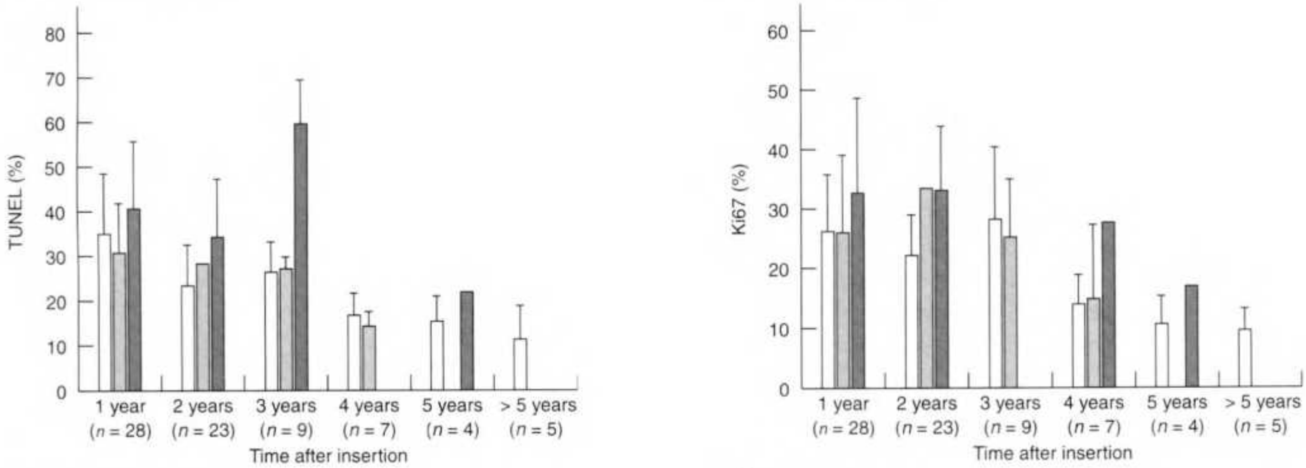
Analysis of the local tissue response indicated a uniform reaction of all patients to the polypropylene mesh. While the quantity and severity of the typical inflammatory reaction varied considerably between patients, the nature of the reaction was identical. Moreover, the tissue response in humans was almost identical to the morphological observations in various animals models[3,5,12,13,15,21]. These data clearly document an increase in the biocompatibility of polypropylene mesh during long-term implantation. Over time, there was a decreased inflammatory reaction and an increase in HSP-70 expression running inversely to the level of inflammation[22]. Even 15 years after implantation, the longest observation in this study, a persisting chronic foreign body reaction could still be detected, implying that mesh is never completely inert with respect to local inflammatory processes.

In conjunction with the increasing biocompatibility during implantation, most of the serious complications



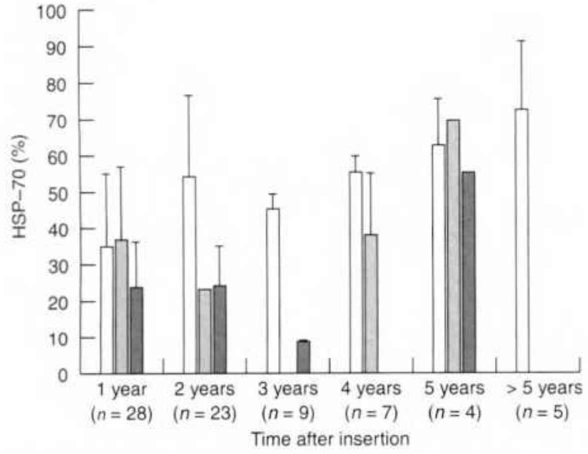
a Inflammatory cells

b Connective tissue



c TUNEL-positive cells

d Ki67



e Heat-shock protein

Fig. 2 Tissue and cellular response to standard polypropylene mesh removed for recurrent hernia, pain or infection according to time after insertion. Values are mean(s.d.). **a** Partial volume of all inflammatory cells, **b** partial volume of connective tissue formation, **c** percentage of TUNEL-positive cells, **d** percentage of Ki67-positive cells and **e** percentage of heat-shock protein (HSP) 70-positive cells

that required reoperation, namely recurrence, chronic pain or infection, occurred within 3 years of implantation. Whether the increased foreign body reaction and inflammatory response in the first 3 years of mesh incorporation is causally related to the observed rate of serious complications, or whether it just reflects the selection of mesh failures for study, remains open to conjecture. Complications 5 years after implantation were extremely rare in all mesh samples recorded in Aachen. However, the delay before explantation of mesh for infection of up to 56 months, for chronic pain of up to 48 months and for recurrence of up to 180 months indicated that in many clinical studies the morbidity rates are underestimated.

Not surprisingly, the tissue response was most affected by concomitant infection, leading to a significantly enhanced inflammatory reaction. However, it was striking that there was little difference in inflammatory response in mesh removed for recurrence or chronic pain, contradicting the possibility of a specific tissue reaction as an underlying cause for either complication.

Acknowledgements

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Original article

Causative factors, surgical treatment and outcome of incisional hernia after liver transplantation

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Background: Little is known about the incidence and causes of herniation, and the results of hernia repair in patients undergoing liver transplantation. Likewise, nothing is known about the best surgical approach for hernia repair.

Methods: A retrospective analysis was conducted of the occurrence of incisional hernia in 290 patients who had liver transplantation between 1990 and 2000, and survived more than 6 months. Follow-up data were obtained from medical records and the outpatient service. Patients were evaluated for various clinical and surgical factors. Hernias were analysed with respect to localization, type of surgical repair and recurrence rate.

Results: Some 17 per cent of the transplanted patients experienced an incisional hernia. Risk factors were acute rejection with affiliated steroid bolus therapy ($P = 0{\cdot}025$), a low platelet count after transplantation ($P = 0{\cdot}048$), and a transverse abdominal incision with upper midline approach ($P = 0{\cdot}04$). Hernias were mainly located at the junction of the transverse and midline incision ($P < 0{\cdot}001$) and the recurrence rate was highest here ($P = 0{\cdot}007$). Prosthetic hernia repair achieved the lowest rate of recurrence and did not increase the incidence of infectious complications.

Conclusion: Improved immunosuppression should avoid early steroid bolus therapy after transplantation. A low platelet count promotes herniation. Transverse abdominal incision seems to be the best approach for liver transplantation. Prosthetic hernia repair does not increase the complication rate.

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Introduction

Incisional hernia is a common complication in conventional abdominal surgery. Several studies have shown that incisional hernias have different aetiologies, which are related to the patient[1], the surgical technique[2], the suture material[3] and the experience of the surgeon[4].

Patients with advanced and chronic liver disease have a high operative risk with increased mortality and morbidity rates[5]. In these patients the abdominal wall is stressed by chronic ascites and a large incision. Patients after liver transplantation therefore represent an excellent group in which to investigate causative factors and analyse the surgical procedure of incisional hernia with regard to major surgery. Although several studies have addressed the problem of incisional hernia in abdominal surgery, none has studied patients after liver transplantation. Furthermore, there is no information available on the effect of different surgical procedures and the techniques of repair in this group of patients.

This study analysed the incidence, causative factors and location of incisional hernias in patients after liver transplantation. The type of surgical repair, incidence of complications and the rate of recurrence were of special interest. Furthermore, the effect of immunosuppression, and the incidence of rejection and rejection therapy were examined in patients after liver transplantation in regard to herniation.

Patients and methods

A total of 290 adult patients who had liver transplantation at the University Clinic of Essen between January 1990 and June 2000 with a postoperative survival of more than

6 months were investigated. Patients were divided in two groups on the basis of postoperative incisional hernia (*n* = 50) or no hernia (*n* = 240) after liver transplantation. All patients were followed up as outpatients in this department. The patients were examined physically and analysed for gender, age, underlying disease, follow-up, Child–Pugh class, diabetes, chronic obstructive pulmonary disease and history of previous abdominal surgery.

The body mass index (BMI = weight (kg)/height $(m)^2$) was measured three times in both groups: (1) at the time of listing for liver transplantation, (2) at the time of liver transplantation and (3) at the time of incisional hernia operation or an equivalent time (18–24 months) after liver transplantation in patients who did not develop an incisional hernia.

The operating time for liver transplantation was documented in each group to estimate the duration of tissue compression effected by the retractor system. The course of total serum bilirubin and the number of platelets were analysed during the first 10 days after operation to determine their impact on the occurrence of incisional hernia. The regimen of immunosuppression and incidence of acute rejection episodes during the first 4 weeks after liver transplantation were also investigated. Only histologically confirmed acute rejection episodes with subsequent steroid treatment were included.

The incidence and type of abdominal incision before liver transplantation was examined, and abdominal reoperations within 1 month of operation and during long-term follow-up after liver transplantation were evaluated. Abdominal reoperations after liver transplantation were always carried out via the incision made during liver transplantation. Patients received a transverse incision with or without an upper midline incision. Closure of the abdominal incision was performed with a running polydioxanone sling suture in one layer using a mass technique. The skin was closed with interrupted monofilament sutures. Wound infection was defined as the secretion of purulent fluid from the incision line with or without surgical intervention and with or without secondary wound healing.

In patients with incisional hernia the following hernia localizations were defined: upper midline incision, right transverse incision, left transverse incision, and junction of upper midline and transverse incision. Incisional hernias were either closed by reapproximation of the fascia or by the Mayo technique, with or without a mesh graft. The suture material was a polydioxanone loop with running closure in the case of fascia reapproximation. In the Mayo technique, absorbable sutures were used. Mesh implants were either absorbable or non-absorbable with the onlay or sublay technique. All patients received subcutaneous wound drainage and antibiotics for 3 days after operation. Recurrence of hernias, their localization and complications were also investigated. Incisional hernias in all but one patient underwent repair in this department.

Statistical analysis of continuous variables was done using *t* test for unpaired data. Frequency data were analysed by χ^2 test with Yates' correction and the Mann–Whitney rank sum test if appropriate.

Results

Causative factors of incisional hernia after liver transplantation

The incidence of incisional hernia after liver transplantation was 17 per cent (*n* = 50). There was no difference in the sex ratio or age between patients who did or did not develop an incisional hernia. Furthermore, there were no statistically significant differences in the distribution of disease, Child–Pugh class, incidence of diabetes and chronic obstructive pulmonary disease, and follow-up time between the groups (*Table 1*).

With regard to the incidence of previous abdominal surgery before liver transplantation, 14 patients (28 per cent) with incisional hernia had experienced a total of 24 interventions, compared with 73 (30 per cent) of patients without a hernia who had undergone a total of 118 interventions (*P* not significant).

Table 1 Incidence of demographic and preoperative risk factors for incisional hernia

	Hernia (*n* = 50)	No hernia (*n* = 240)
Sex*		
Men	29 (58)	139 (58)
Women	21 (42)	101 (42)
Age (years)†	47 (22–65)	46 (19–68)
Mean follow-up (months)†	58 (9–132)	51 (7–134)
Liver disease*		
Hepatitis	21 (42)	92 (38)
Alcoholic	11 (22)	41 (17)
Tumour	3 (6)	6 (3)
Cryptogenic	7 (14)	17 (7)
Autoimmune PSC, PBC	5 (10)	50 (21)
Acute liver failure	1 (2)	16 (7)
Budd–Chiari syndrome	2 (4)	6 (3)
Other	0 (0)	12 (5)
Child grade*		
A	9 (18)	47 (20)
B	29 (58)	138 (58)
C	12 (24)	55 (23)
Diabetes*	8 (16)	41 (17)
Chronic obstructive pulmonary disease*	3 (6)	12 (5)

*Values in parentheses are percentages; values are †mean (range). PSC, primary sclerosing cholangitis; PBC, primary biliary cirrhosis

Although obesity is rare in liver cirrhosis, many of these patients experienced a marked variation in body-weight owing to the treatment of ascites and during the operative procedure of liver transplantation. In addition, chronic ascites caused a continous mechanical stress on the abdominal wall. Besides the Child level, the body-weight was investigated in both groups at three different timepoints in order to evaluate its influence on the development of incisional hernia. BMI was measured at the time of listing for liver transplantation and at the time of transplantation. The third BMI measurement was related to the date of incisional hernia repair. In the hernia group, the mean(s.d.) interval between liver transplantation and hernia operation was 22(16) months. Patients without incisional hernia were analysed for BMI between 18 and 24 months after liver transplantation. In patients with a shorter follow-up, the BMI at the time the patient was last weighed was calculated. Comparison of BMI between the two groups revealed no significant differences. Moreover, the mean BMI did not show any variation within each group between the three measurements (*Table 2*).

Exposure of the upper abdomen during liver transplantation requires a huge wound retractor system, which leads to long-term compression of the incisional margin during the procedure. To verify whether this stress on the wound margin was involved in the pathogenesis of incisional hernia after liver transplantation, the duration of operation was examined; however, the operation time was similar in both groups.

To evaluate whether any clinical factors or wound healing affected the occurrence of incisional hernia, total serum bilirubin, the number of platelets, the type of immunosuppression and the incidence of histologically confirmed acute rejection were evaluated. Neither the total serum bilirubin level nor the immunosuppressive regimen (dual, triple or quadruple therapy) showed a remarkable difference between the two groups. In contrast, the mean platelet count during the first 10 days after liver transplantation was significantly lower ($P = 0{\cdot}048$) in patients with subsequent incisional hernia than in patients without herniation. Platelet replacement was similar in both groups (data not shown). Furthermore, the incidence of early acute rejection episodes after liver transplantation with steroid treatment was significantly higher ($P = 0{\cdot}025$) in patients with incisional hernia (*Table 2*).

All patients with incisional hernia received a transverse incision with an upper midline incision. In contrast, no patient with a transverse abdominal incision only experienced an incisional hernia after liver transplantation ($P = 0{\cdot}04$). The frequency of abdominal reoperation after liver transplantation was analysed as a further risk factor. This analysis distinguished early reoperations (during the first month) and those in the long term after liver transplantation. There was no notable difference in the

Table 2 Incidence and distribution of intraoperative and postoperative risk factors for incisional hernia

	Hernia ($n = 50$)	No hernia ($n = 240$)
Body mass index*		
At liver transplantation listing	23·5 (15·6–32·4)	24·0 (16·0–35·4)
At liver transplantation	23·8 (16·3–30·5)	24·2 (13·8–36·7)
At hernia repair or equivalent follow-up	24·1 (15·6–32·4)	24·6 (15·1–37·4)
Liver transplantation operating time (min)*	331 (160–540)	328 (170–700)
Total serum bilirubin (mg/dl)†	8·5(5·7)	8·0(5·7)
Platelets (per nanolitre)†	112(54)§	151(78)
Immunosuppression regimen‡		
Dual	5 (10)	24 (10)
Triple	30 (60)	163 (68)
Quadruple	15 (30)	53 (22)
Patients with acute graft rejection‡	27 (54)¶	86 (36)
No. of episodes of acute graft rejection	32	114
Incision type‡		
Transverse with upper midline	50 (100)	216 (90)
Transverse	0 (0)**	24 (10)
Relaparotomy‡		
Within 1 month after transplantation	12 (24)	48 (20)
Long period after transplantation	8 (16)	26 (11)
Wound infection‡	8 (16)	24 (10)

*Values are mean (range); †values are mean(s.d.); ‡values in parentheses are percentages. §$P = 0{\cdot}048$ (Mann–Whitney rank sum test), ¶$P = 0{\cdot}025$, **$P = 0{\cdot}04$ *versus* no hernia (χ^2 test) frequency of reoperation between periods in either group. There was a slightly higher incidence of wound infection in patients with incisional hernia, which did not reach statistical significance (*Table 2*).

Localization, surgical treatment and outcome of incisional hernia after liver transplantation

Incisional hernias were repaired between 4 and 73 months after liver transplantation. A significantly higher proportion of incisional hernias were located at the junction of the upper midline and transverse incision than at other locations ($P < 0{\cdot}001$). Two hernias were found in six patients in this group (12 per cent). Hernias were not repaired in six patients either because of the patients' poor clinical condition or death.

Thirty of the hernias were closed by reapproximation of the fascia or by the Mayo technique. The remainder were closed by combination of a mesh with one of the above procedures (*Table 3*). Recurrent hernias were seen in 11 patients. In five of these patients additional hernias were localized. Furthermore, in seven of the 11 patients with recurrent hernia, the initial and recurrent hernias were localized at the junction of the midline and the transverse incision ($P = 0{\cdot}007$ *versus* other locations). In nine patients, reoperation of the recurrent hernia took place after 2–17 months. Hernia repair was performed in three patients by reapproximation of the fascia and in two patients with a mesh. In the remaining four patients, hernia repair was performed by the Mayo technique (one) and with a mesh (three). Of these nine patients with hernia recurrence, four had a further recurrent hernia, and a different repair technique was used in each patient.

Table 3 Localization, treatment and outcome of incisional hernia

	No. of patients	No. with recurrence
Localization ($n = 50$)		
Junction of transverse and upper midline incision	30 (60)*	7†
Upper midline incision	6 (12)	2
Right transverse incision	8 (16)	1
Left transverse incision	6 (12)	1
Surgical procedure ($n = 44$)		
Fascia reapproximation	17 (39)	4
Fascia reapproximation with mesh	8 (18)	1
Mayo	13 (30)	5
Mayo with mesh	6 (14)	1

Values in parentheses are percentages. $^*P < 0{\cdot}001$, $^‡P = 0{\cdot}007$ *versus* other locations (χ^2 test)

Hernia recurrence was seen most frequently with the Mayo procedure. With reference to the initial hernia repair, application of a mesh led to a significantly lower incidence of hernia recurrence than the other techniques ($P = 0{\cdot}011$). Overall, 14 patients received a mesh repair. An absorbable mesh was used in seven patients and the remainder received a non-absorbable mesh. Among the 44 patients who underwent 57 operations for hernia repair, the incidence of complications was 2 per cent (one patient). This patient, who received a mesh for incisional hernia repair, experienced an episode of infection that required reoperation.

Discussion

Incisional herniation after abdominal surgery is an ongoing challenge in surgery. Nevertheless, there is still a lack of data on patients with chronic advanced liver disease who develop incisional hernia after major surgery. The present study analysed the causative factors for herniation and the influence of variables related to patients with or without cirrhosis after liver transplantion.

Male gender, increased age, disease, diabetes, chronic obstructive pulmonary disease and postoperative wound infection could not be verified as risk factors for herniation in patients after liver transplantation, as confirmed by others with heterogeneous patient cohorts[6,7]. The occurrence of ascites in patients with cirrhosis stresses the morphological integrity of the abdominal wall[8,9]. Furthermore, there is a relationship between increased BMI and incisional hernia[10,11]. In the present study, therefore, the Child–Pugh class and the course of BMI were evaluated with regard to incisional hernia. There was no correlation between the incidence of preoperative ascites as shown by the Child level and/or increased BMI and incisional herniation after liver transplantation. Although the morphological integrity of the abdominal wall was affected by the incidence of previous abdominal surgery[12], the present data excluded this as a risk factor for incisional hernia. In contrast to an earlier study that identified reincision as a significant risk factor[13], it did not matter whether liver transplantation was done via a previous incision or with an extension to the former abdominal approach. The elimination of jaundice as a result of successful liver transplantation, or the authors' vigorous policy of removing the entire scar tissue at the time of liver transplantation (as noted by others[14]), might explain this discrepancy. In addition, neither mechanical alterations during liver transplantation by the wound retractor nor reoperations during the early or later period after liver transplantation were risk factors for the occurrence of incisional hernia in this study.

An increase in total serum bilirubin level after liver transplantation has been described, which may affect wound healing[15,16]. However, the same level of hyperbilirubinaemia was present in both groups in this study, eliminating this as a risk factor for the occurrence of incisional hernia. This was in contrast to a study in which an increased incidence of incisional hernia was observed in jaundiced patients with raised total serum bilirubin due to malignant disease[17]. The association with malignant disease may be responsible for this different finding, as only 4 per cent of the present patients suffered from malignancy. Furthermore, successful liver transplantation with subsequent improvement of liver function as well as temporary jaundice may explain these contrasting findings.

Patients with liver cirrhosis suffer from chronic thrombocytopenia[18], which may also affect wound healing[19]. Early after liver transplantation, patients with incisional hernia had a significantly lower platelet count. This finding cannot be explained by an effect of immunosuppression on platelet function and wound healing[20] as the pattern of immunosuppressive therapy was similar in both groups. However, a deficiency in thrombopoietin production and generation of platelets[21] in the liver graft could explain the low platelet count in the hernia group. Graft dysfunction due to acute rejection episodes in this group, strengthened by the steroids given in antirejection therapy, could explain this deficiency. Furthermore, the deleterious effect of steroids on wound healing is well known[22,23]. The authors therefore propose use of a more potent immunosuppressive regimen to avoid acute rejection episodes in the early postoperative phase. In contrast, the use of chronic steroid therapy before liver transplantation was more common in patients without incisional hernia (10 *versus* 21 per cent). This excludes the chronic use of steroids before liver transplantation as a risk factor for herniation. This observation was in accordance with data from non-transplanted patients[24].

Several studies have mentioned the type of abdominal incision as a risk factor for herniation[25–27]. In this study, only patients with a combined incision experienced an incisional hernia ($P = 0{\cdot}04$). Studies of patients with cirrhosis who are in need of abdominal surgery confirm this result and indicate a preference for a transverse abdominal incision[28]. Technical failure in abdominal closure was unlikely to explain this finding since liver transplantation was always performed by a small group of senior surgeons, which minimized the incidence of herniation[29]. Although there are no published data on the link between the combined type of abdominal incision and incisional hernia, several studies[30,31] have identified the midline incision as a risk factor for herniation after liver transplantation. In the present series 66 per cent of hernias involved the upper midline incision. In accordance with other data[32], the highest rate of hernia recurrence was also at this site. The hernia recurrence rate in the present study was 22 per cent, lower than that reported previously[33–35].

Hernia recurrence was not significantly related to the operative technique used, although there were more hernia recurrences after the Mayo procedure, as reported previously[36,37]. Addition of an absorbable or non-absorbable mesh graft to either closure technique was of major benefit. This approach achieved the lowest rate of hernia recurrence in this and other[38–40] series. However, the use of prosthetic material is controversial in transplanted patients because of the risk of serious infection in an immunosuppressed individual, although several studies have reported no increase in complications among non-transplanted patients who have undergone prosthetic hernia repair as long as major conditions are considered[38,41,42]. Nevertheless, owing to limited data, the use of meshes in transplanted patients is still under restriction. In the present series, only one patient who received a mesh hernia repair suffered an infection. This low incidence of complications was achieved by the use of perioperative antibiotic prophylaxis, as described by others[43]. To reduce the risk of hernia recurrence, the authors therefore favour incisional hernia repair with prosthetic material after liver transplantation.

In conclusion, in the present study the occurrence of acute rejection episodes and steroid pulse therapy, and a low platelet count in the early postoperative period were major risk factors for incisional hernia after liver transplantation. The upper midline incision was identified as a significant risk factor for incisional herniation in this group of patients, and a transverse approach should therefore be preferred. A clear advantage of the primary use of mesh for hernia repair in patients under immunosuppression was shown, with a lower rate of hernia recurrence and no increase in the incidence of complications.

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Original article

Microemulsion cyclosporin inhibits vascular remodelling and attenuates associated changes in profibrotic gene expression in an experimental model of allograft vasculopathy

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Background: Chronic allograft dysfunction (CAD), the leading cause of solid organ transplant failure, is characterized by histological evidence of extracellular matrix (ECM) accumulation (fibrosis). The aim of this study was to characterize the changes in fibrosis-associated gene expression in an experimental model of CAD and to measure the effect of the immunosuppressant cyclosporin on these changes.
Methods: Lewis recipients of F344 rat thoracic to abdominal transplants were administered cyclosporin or no treatment. Vascular remodelling and ECM accumulation (picrosirius red) were measured using computerized histomorphometry. Fibrosis-associated gene expression was studied by semiquantitative reverse transcription–polymerase chain reaction.
Results: Cyclosporin inhibited medial ECM accumulation and vascular remodelling in allografts. This was associated with an attenuation of the graft inflammatory infiltrate and a reduction in intragraft matrix metalloproteinase (MMP) 2 and MMP-9 messenger RNA (mRNA) levels. There was a significant negative correlation between neoadventitial ECM density and MMP-9 expression, as well as with vessel circumference. Neoadventitial ECM density was significantly higher in the cyclosporin-treated group than in animals with untreated allografts, as were mRNA levels of collagen 3 and tissue inhibitor of metalloproteinase 1.
Conclusion: The alloimmune injury itself may contribute directly to vascular remodelling and fibrosis in allograft vasculopathy. Cyclosporin attenuated this component of the pathophysiology of CAD effectively.

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Introduction

Chronic allograft dysfunction (CAD) is a leading cause of late allograft loss; currently available immunosuppressants have little or no effect on the progression of this condition in clinical transplantation[1,2]. CAD is defined histologically by a chronic fibroproliferative tissue response to injury that includes allograft vasculopathy and parenchymal extracellular matrix (ECM) accumulation (fibrosis)[3–5]. Allograft vasculopathy is characterized by neointimal hyperplasia and vascular remodelling, pathological processes that are associated with changes in the regulation of ECM turnover. Central to these processes in many models of vascular injury are the matrix metalloproteinases (MMPs), a family of zinc-dependent proteases that can collectively break down all the constituents of the ECM[6]. The activity of these proteases in turn is tightly regulated by a highly specific family of inhibitory proteins, the tissue inhibitors of metalloproteinases (TIMPs). The role of MMPs and TIMPs in the development of allograft vasculopathy remains to be defined.

The aim of this study was to characterize the changes in gene expression that are important in regulating ECM turnover in an animal model of CAD and to assess the effect of the immunosuppressant cyclosporin on these changes.

Materials and methods

Experimental model

The thoracic aorta to abdominal aorta allograft model described by Mennander *et al.*[7] was used. A segment of the descending thoracic aorta approximately 3 cm in length was excised, perfused thoroughly with phosphate-buffered saline and used as a transplant. F344 rats acted as donors and recipients, and Lewis rats as recipients. Ischaemic injury to the graft was minimized by immersion in an ice bath at 4°C between procedures. Through a midline laparotomy incision, the segment of thoracic aorta was anastomosed end to end to the recipient abdominal aorta using 9/0 polypropylene sutures. The graft was transplanted into a heterotopic position below the renal arteries and above the aortic bifurcation, forming a loop in the recipient abdomen.

Animals were kept in a controlled environment with unlimited access to feed and water. Graft harvest was performed in heavily anaesthetized rats, after which the animal was killed by exsanguination.

Drugs

Microemulsion cyclosporin (Neoral; Novartis, Basle, Switzerland) was prepared as a solution of 5 mg/ml in olive oil, stored at 21°C, and administered orally by gavage.

Experimental groups

In group 1 animals (isografts; negative control), thoracic aorta was transplanted from one F344 rat to another.

In group 2 (allografts; positive control), thoracic aorta was transplanted from F344 rats to Lewis rats receiving cyclosporin 5 mg/kg daily, orally by gavage, for 14 days only, followed by no immunosuppression until death.

In group 3, thoracic aorta was transplanted from F344 rats to Lewis rats receiving microemulsion cyclosporin 5 mg/kg daily, orally by gavage, from the day of transplantation until death.

In this experiment, six rats in each group were killed at each of weeks 4, 8, 12 and 16, with a further six rats in group 1 killed at 24 weeks.

Histological analysis

Segments of the aortic graft were embedded in paraffin wax and multiple sections were stained with haematoxylin and eosin. The level of inflammatory infiltrate was assessed semiquantitatively by a blinded single observer and categorized as follows: none, no inflammatory cells visualized; mild, inflammatory cell localized to the endothelium; moderate, dense inflammatory cell infiltrate in the innermost layer of the vessel wall; severe, dense inflammatory cell infiltrate throughout all layers of the vessel wall.

The vessel circumference and area fraction of the intima and media were quantified using a computer image analysis system. The intima was defined as the area between the endothelium and the internal elastic lamina, and the media as the area between the internal and external elastic laminas. Paraffin-embedded sections were also stained with picrosirius red, and the area fraction of ECM staining was quantified by means of computerized histomorphometry[8].

Determination of allograft gene expression with reverse transcription–polymerase chain reaction

Total messenger RNA (mRNA) was extracted from aortic tissue and complementary DNA (cDNA) molecules were synthesized by reverse transcription (RT). These cDNA species were amplified by polymerase chain reaction (PCR) and quantified in an enzyme-linked immunosorbent assay (ELISA) system. Relative quantification was performed by comparison of the signal intensity to that of the housekeeping gene β-actin. These techniques have been described in more detail elsewhere[9]. Probe and primer sequences used in these experiments have also been published previously[10].

Substances to be assessed

To take into account all the factors that influence smooth muscle cell proliferation, migration and deposition of ECM during neotima formation, the following species were studied with RT-PCR: MMP-2, MMP-9, TIMPs 1, 2 and 3, transforming growth factor (TGF) β and collagen 3.

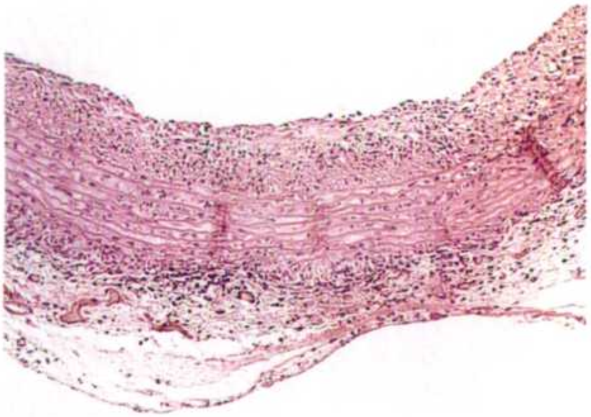
Analysis of results

The levels of gene expression were compared in the different study groups using non-parametric statistical analysis. Allograft gene transcripts for matrix proteins and growth factors were correlated with intimal thickening, expressed as the intima : media ratio; with vascular remodelling, expressed as vessel circumference (of the internal elastic lamina); and with collagen accumulation, expressed as a product of the percentage mean media picrosirius red staining and the medial area. Statistical analysis was performed using the Statistical Package for the Social Sciences version 8·0 (SPSS, Chertsey, UK).

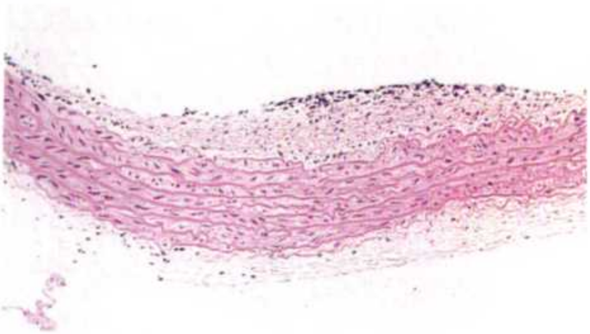
Results

Histological analysis

By 4 weeks after transplantation there was a dense inflammatory infiltrate in allografts, in many cases transmural, that was not seen in isografts (*Fig. 1*); this infiltrate was also present at 8, 12 and 16 weeks. By 12 weeks early



a Untreated



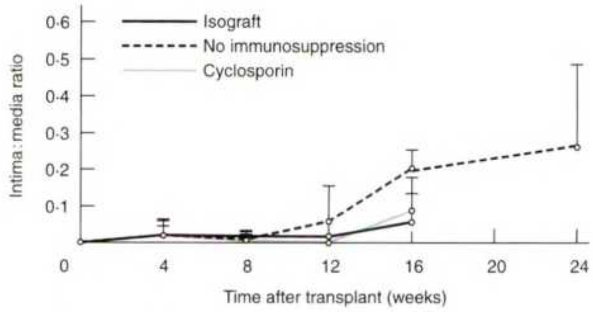
b Cyclosporin

Fig. 1 Representative photomicrographs of haematoxylin and eosin-stained section of rat aorta. **a** Untreated allografts have a dense transmural inflammatory infiltrate associated with intimal and medial thickening. **b** These changes were attenuated by cyclosporin. (Original magnification × 40)

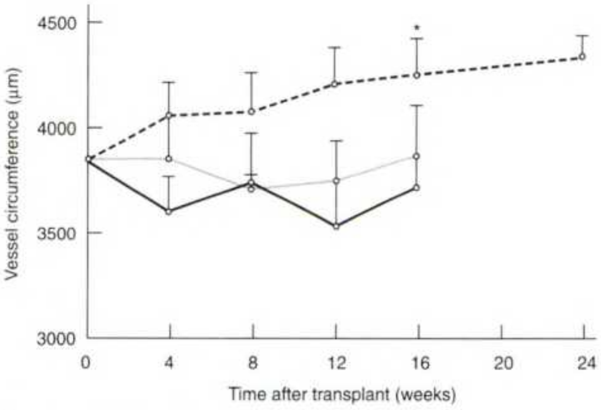
intimal hyperplasia was evident in untreated allografts and by 16 weeks intimal thickening appeared to have slowed such that there was no significant difference between the intima : media ratio at 16 and 24 weeks in untreated allografts (*Fig. 2a*). Expansive vascular remodelling occurred in a linear fashion between 4 and 24 weeks (*Fig. 2b*). Cyclosporin significantly inhibited both intimal hyperplasia (mean(s.d.) intima : media ratio 0·08(0·09) *versus* 0·20(0·05); P = 0·01, Mann–Whitney U test) and expansive vascular remodelling (mean(s.d.) internal elastic lamina circumference 3·8(0·2) *versus* 4·2(0·2) mm; P = 0·02) compared with untreated allografts, as well as significantly attenuating the severity of the inflammatory response within the graft at 4 weeks (*Fig. 3*) and at all subsequent timepoints (data not shown).

Results of picrosirius red histomorphometry

There was increased medial ECM accumulation within the allografts relative to isografts at 16 weeks (mean(s.d.) area fraction stained 0·45(0·01) *versus* 0·35(0·03) mm^2; P = 0·03, Mann–Whitney U test). This was not associated with a change in ECM density but was due to an increase in medial area. Allograft medial ECM accumulation was significantly reduced by cyclosporin (0·38(0·04) mm^2; P = 0·01).



a Intima : media ratio



b Vessel circumference

Fig. 2 Effect of cyclosporin on allograft vasculopathy. Values are mean(s.d.) **a** Intima : media ratio. Neointima formation occurs between 8 and 16 weeks in untreated allografts relative to isografts. Intimal thickening was significantly inhibited by cyclosporin (P = 0·02, Mann–Whitney U test). **b** Vessel circumference. Allografts underwent significant progressive expansive remodelling between 0 and 24 weeks. This process was inhibited by cyclosporin; *P = 0·01 (Mann–Whitney U test).

Very little ECM staining was detected in the neointima at 16 weeks, and so this was not included in the analysis. As this would predominantly have increased the total ECM content in the untreated allograft group, however, it would not have altered the results greatly. In the present model it was impossible accurately to distinguish true allograft adventitia from peritoneal adhesions, and therefore neo-adventitial thickness or ECM content was not assessed.

Adventitial ECM density, however, was reduced in allografts relative to isografts (0·72(0·07) *versus* 0·85(0·07); P = 0·05, Mann–Whitney U test) and this effect was attenuated by the administration of cyclosporin (0·80(0·05); P = 0·08).

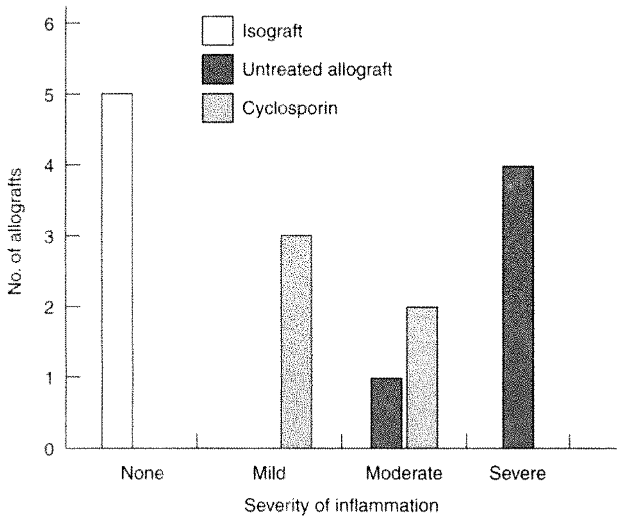


Fig. 3 Inflammatory infiltrate 4 weeks after transplantation. At 4 weeks there was a significant difference in the severity of the inflammatory infiltrate between isografts, untreated allografts and cyclosporin-treated allografts ($P < 0·001$, χ^2 test)

Results of reverse transcription–polymerase chain reaction enzyme-linked immunosorbent assay

MMP-9 mRNA transcript levels were increased in untreated allografts relative to isografts at 4 weeks, and this increase was attenuated by cyclosporin (data not shown). There was no significant difference between the groups for the other genes studied at 4 weeks. Gene mRNA : β-actin mRNA ratios for fibrosis-associated genes studied at subsequent timepoints are listed in *Table 1*. There was no apparent difference between the groups for TIMP-2 or TIMP-3 at any timepoint (data not shown). TIMP-3, however, correlated closely with MMP-2 at all timepoints; for example, at 8 weeks, $r_s = 0·8$, $P < 0·001$.

At 8 weeks there was a reduction in TIMP-1 and collagen 3 gene expression in untreated allografts relative to isografts, with a persistent significant increase in MMP-9 transcript levels in untreated allografts relative to isografts. When gene : actin ratios were correlated, a significant negative correlation was observed between MMP-9 and collagen 3 ($r_s = -0·6$, P = 0·01). Cyclosporin significantly

Table 1 Enzyme-linked immunosorbent assay values of reverse transcription–polymerase chain reaction complementary DNA product of messenger RNA species isolated from rat aorta using Dynabead extraction at different timepoints

	Time after transplantation (weeks)		
Gene	8	12	16
MMP-2			
Isograft	1·32(0·28) (0·97, 1·67)	1·22(0·29) (0·86, 1·58)*	1·35(0·66) (0·29, 2·40)
Allograft	1·01(0·24) (0·76, 1·27)	1·70(0·25) (1·29, 2·10)	1·63(0·43) (1·18, 2·09)
Cyclosporin	0·84(0·21) (0·62, 1·07)	1·14(0·26) (0·87, 1·42)*	1·25(0·81) (0·50, 2·00)
MMP-9			
Isograft	0·66(0·30) (0·30, 1·03)†	1·41(0·55) (0·73, 2·10)†	0·81(0·19) (0·51, 1·11)†
Allograft	1·99(0·58) (1·38, 2·60)	2·45(0·72) (1·56, 3·34)	2·28(0·25) (2·01, 2·55)
Cyclosporin	1·20(0·28) (0·91, 1·49)*	2·56(0·56) (1·97, 3·15)	1·87(0·28) (1·61, 2·13)*
Collagen 3			
Isograft	0·97(0·10) (0·85, 1·09)*	1·74(0·38) (1·27, 2·21)	3·62(0·83) (2·30, 4·94)*
Allograft	0·79(0·05) (0·74, 0·84)	1·97(0·48) (1·37, 2·57)	2·29(0·47) (1·70, 2·88)
Cyclosporin	0·87(0·12) (0·74, 1·00)†	1·66(0·27) (1·38, 1·94)	2·03(0·18) (1·86, 2·20)
TGF-β			
Isograft	1·49(0·16) (1·29, 1·69)	2·01(0·52) (1·37, 2·67)*	4·66(0·75) (2·80, 6·52)*
Allograft	1·37(0·12) (1·24, 1·49)	2·83(0·43) (2·30, 3·36)	4·73(0·17) (4·55, 4·91)
Cyclosporin	1·12(0·10) (1·02, 1·22)†	3·02(0·53) (2·46, 3·58)	4·08(0·49) (3·63, 4·53)
TIMP-1			
Isograft	1·00(0·12) (0·85, 1·16)*	1·57(0·18) (1·35, 1·79)	1·62(0·11) (1·44, 1·80)*
Allograft	0·86(0·05) (0·80, 0·91)	1·89(0·35) (1·45, 2·32)	1·35(0·15) (1·17, 1·54)
Cyclosporin	0·81(0·10) (0·70, 0·92)	1·56(0·25) (1·30, 1·82)	1·13(0·01) (1·05, 1·12)†

Values are mean(s.d.) (95 per cent confidence interval) ratios for messenger RNA of the gene specified to that of the housekeeping gene β-actin. MMP, matrix metalloproteinase; TGF, transforming growth factor; TIMP, tissue inhibitor of matrix metalloproteinase. *$P < 0·05$, †$P < 0·01$ *versus* allograft (Mann–Whitney U test) attenuated the MMP-9 rise and the collagen 3 fall, as well as significantly reducing TGF-β mRNA transcript levels (*Table 1*). MMP-2 expression was also reduced by cyclosporin at 8 weeks, but this was not statistically significant (data not shown).

In aortas analysed after 12 weeks there was a significant increase in MMP-2, TGF-β and MMP-9 expression in untreated allografts relative to isografts. Cyclosporin inhibited MMP-2 transcription in allografts. There were no significant differences with respect to profibrotic genes such as collagen 3 and TIMP-1 at this timepoint.

At 16 weeks there were significant reductions in the expression of TIMP-1 and collagen 3, as well as a significant increase in MMP-9 in untreated allografts relative to isografts; however, these later changes in collagen 3 and TIMP-1 expression were not attenuated by cyclosporin.

Histological correlates of observed changes in matrix metalloproteinase 9 gene expression

MMP-9 transcript levels in the vessel wall increased with increasing histological severity of the inflammatory infiltrate (*Fig. 4*). Gene : actin ratios for MMP-9, as determined by ELISA, were also shown to correlate significantly with medial collagen content ($r_s = 0.7$, $P = 0.02$), vessel circumference ($r_s = 0.8$, $P < 0.001$) and intimal area ($r_s = 0.6$, $P = 0.02$). There was a weak but statistically significant negative correlation between MMP-9 transcript levels and neoadventitial collagen density ($r_s = 0.5$, $P = 0.05$).

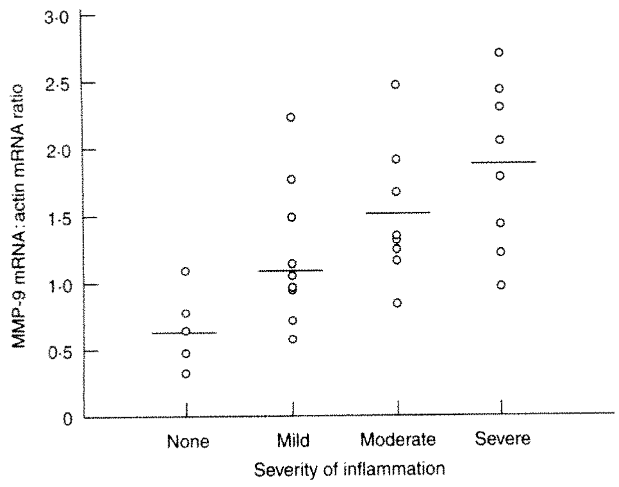


Fig. 4 Matrix metalloproteinase (MMP) 9 expression *versus* severity of the inflammatory infiltrate. Bars denote mean values. mRNA, messenger RNA. There was a significant difference between the mean values ($P = 0.001$; Kruskall–Wallis test)

Discussion

These data show that the development of vasculopathy in rat aortic allografts was associated with progressive expansive vascular remodelling, inflammation, increased medial ECM accumulation and a reduction in adventitial collagen density. These changes were associated with increased intragraft MMP-9 and decreased TIMP-1 and collagen 3 mRNA levels compared with negative controls (isografts). Inhibition of graft inflammation by cyclosporin attenuated both the increase in gelatinase expression in allografts and the changes in profibrotic gene activity, and significantly inhibited neointima formation, medial collagen accumulation and expansive vascular remodelling.

Expansive remodelling in rat allografts correlated with MMP-9 gene expression. An association between MMP-9 and expansive vascular remodelling is also seen in balloon injury and high shear stress models of vascular injury[11–13]; however, the molecular basis of this process remains obscure. In rat allografts MMP-9 transcript levels were associated with severity of the inflammatory infiltration in the grafts. MMP-9 is expressed by infiltrating T cells and activated macrophages *in vivo* and *in vitro*[14], whereas in mice aortic allografts MMP-9 is localized to inflammatory cells throughout the allograft, but particularly the adventitia[15]. MMP-9 mRNA levels also correlated inversely with adventitial collagen density. Metalloproteinase activity may promote expansion by degradation of adventitial collagen[16], as a collagen-dense adventitia may represent a mechanical barrier to vessel enlargement. Adventitial fibroblast collagen production is a critical determinant of constrictive remodelling in balloon-injured rabbit arteries[17], and it is noteworthy that expansive remodelling was associated with decreased collagen 3 and TIMP-1 expression in the present model. Remodelling could also arise as a response to degradation of elastin, a substrate for MMP-9 and a major structural component in these vessels (elastin lamellae). Cyclosporin, by attenuating the T-cell response and subsequent macrophage infiltration, inhibited expansion. These data suggest that vascular remodelling in allografts is not solely a component of the tissue response to injury[18] but may in part be mediated by the alloimmune injury itself.

Neointimal thickening between 12 and 16 weeks coincided with significant increases in MMP-2, TGF-β and MMP-9 expression. Similar changes in gene expression are associated with ECM turnover during formation of the neointima in other experimental models[19–22], and these changes may underly smooth muscle cell migration and proliferation in the aortic allografts. MMP-9 was not inhibited by cyclosporin at 12 weeks compared with untreated allografts. This is unexplained; however, the severity of inflammatory infiltrate within the untreated allografts at 12 weeks appeared less than at other timepoints, with subsequently lower MMP-9 transcription in the untreated allografts.

A beneficial effect of cyclosporin on allograft vasculopathy is also apparent in clinical heart transplantation. Two recent intracoronary ultrasound studies have suggested that high-dose cyclosporin may have a beneficial effect on the development of CAD. In one cross-sectional study a positive correlation was found between a coronary intimal thickness greater than 3 mm and a lower daily cyclosporin dose[23]. In another multicentre study[24] patients receiving more than 5 mg/kg cyclosporin daily had significantly less development of CAD at 1 year than those receiving a daily dose of less than 3 mg/kg. Both clinical and experimental evidence, therefore, suggests that the success of cyclosporin in heart transplantation is limited by unacceptable side-effects at larger doses, but that in an appropriate dose cyclosporin may attenuate CAD.

Allograft medial fibrosis was reduced by the administration of cyclosporin, in association with a reduction in graft inflammation. In apparent contradiction, there was no increase in profibrotic genes in allografts relative to isografts at any timepoint. This can be explained by several factors. The observation that levels of TIMP-1 and collagen 3 did not appear to be raised during ECM accumulation and intimal vascular smooth muscle cell proliferation in rat allografts may indicate that this study failed to examine the distribution of mRNA or protein throughout the vessel wall. It is possible that a large reduction in TIMP or collagen mRNA levels in, for example, the adventitia may mask different and more subtle changes in other parts of the vessel wall. Furthermore, although TIMP-1 was expressed constitutively in uninjured rat carotid arteries[21] and levels were detected in isografts in this model, TIMP-1 expression was not increased during periods of ECM (collagen) accumulation following balloon injury in the rat carotid artery[21]. TIMP-1, therefore, may not be as important in determining the rate of ECM accumulation in the rat as in other species[19,22,25]. Picrosirius red stains not only collagen but also many other ECM components[26], and it is possible that the observed medial fibrosis involved the accumulation of predominantly non-collagen molecules.

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Original article

Laparoscopic transperitoneal procedure for routine repair of groin hernia

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Background: Laparoscopic transperitoneal hernioplasty (TAPP) repair of inguinal hernias is thought to be a difficult surgical technique with high complication rates. The present study evaluated TAPP based on prospective documentation.

Methods: The primary aim of the study was analysis of the individual learning curve, comparing consultants with trainees. Secondary endpoints included postoperative morbidity, time of disability and rate of recurrence.

Results: A total of 8050 TAPP repairs have been performed since 1993. By 2001, 99·9 per cent of all hernia repairs were done by TAPP. The median operation time dropped from 50 min in the first 600 cases to 42 min thereafter. The morbidity rate decreased from 9·3 to 2·6 per cent, and the rate of recurrence from 4·8 to 0·4 per cent. Within the same interval the proportion of training procedures increased from 1·7 to 44·9 per cent in 2001. Morbidity and recurrence rates were similar for trainees and consultants.

Conclusion: TAPP is an effective and safe technique. It can be performed in a standard way for all inguinal and femoral hernias. The present results indicate that TAPP is possible in a routine setting, as well as in the training situation for young surgeons.

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Introduction

The introduction of endoscopic techniques has added a new dimension to groin hernia surgery. In accordance with the Pascal principle[1], and similar to the anterior mesh repair of Lichtenstein, this new technique allows tension-free repair of the hernia defect. Randomized studies have shown significant improvements in postoperative pain and rehabilitation rates in comparison to suture repair done by the Shouldice or Lichtenstein techniques[2,3]. These benefits come with higher operation costs, a greater degree of difficulty and correspondingly longer operation times. Critics also maintain that the laparoscopic–endoscopic technique can be performed safely only by specialist surgeons on selected patients[4].

This study was a prospective analysis of data from a large and completely unselected group of consecutive hernia repairs, which aimed to assess the feasibility of laparoscopic transperitoneal hernioplasty (TAPP) in the clinical routine of a non-specialist teaching hospital, and to assess its efficacy with respect to the different hernia types.

Patients and methods

Laparoscopic hernioplasty via a transabdominal technique with preperitoneal placement of a polypropylene mesh (TAPP) was first introduced in this department in April 1993. Since then, open hernia repair has increasingly been replaced by TAPP, with an increasing total number of hernia repairs (*Fig. 1*). In the past 6 years more than 1000 hernia repairs have been carried out annually, 98·1 per cent using the laparoscopic technique. An open procedure without mesh (Shouldice) was performed only on patients aged under 20 years and in those who declined mesh. An open procedure with mesh (Lichtenstein) was performed on elderly patients with a contraindication to general anaesthesia. All data relating to individual patients and operations were collected prospectively and entered into a database. Results reported in this publication represent the status of the database on 1 March 2002.

Surgical technique

The operating technique for TAPP has been published previously[5–8]. After the first 600 operations (learning and method development) technical modifications were introduced, including replacement of the sharp three-edged, filed, disposable trocars with blunt conical reusable trocars. Currently three reusable trocars are used (10 mm at the umbilicus with 30° optic, 12 mm right lateral medioclavicular and 5 mm left lateral). A larger polypropylene mesh is now used (increased from 8 × 12 cm initially to

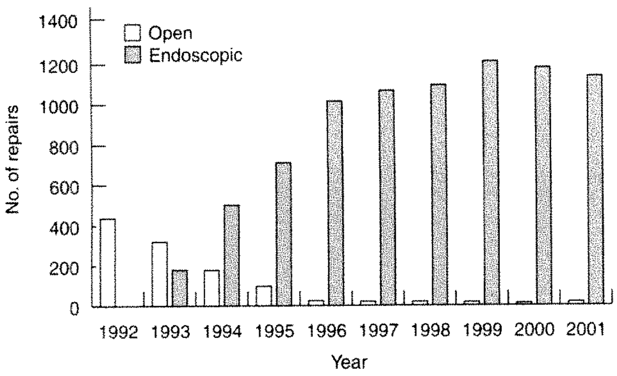

Fig. 1 Number of open (Shouldice, Lichtenstein) and endoscopic (laparoscopic transperitoneal hernioplasty) inguinal hernia repairs in the Department of Surgery, Marienhospital Stuttgart, 1992–2001

10 × 15 cm) and the number of clips (B shaped) has been reduced to a maximum of four to six (two at Cooper's ligament, two at the rectus medial to the epigastric vessels, two on the fascia transversalis craniolateral to the epigastric vessels) with strict avoidance of clips in the area distal to the ileopubic tract. The peritoneum is closed with a continuous suture and, since about the 2000th procedure, unslitted mesh has been used almost exclusively. The importance of complete dissection of the medial compartment of the peritoneum as well as wide dissection of the peritoneum from the spermatic cord cranially to about mid-psoas has been recognized. This is an important requirement for wrinkle-free placement of a large piece of mesh.

All operations were carried out under general anaesthesia; a urinary catheter was not inserted routinely. When the bladder was found to be full at operation, it was drained suprapubically. All patients received a single perioperative dose of prophylactic antibiotic. On the evening of the operation patients were encouraged to visit the toilet with assistance and could drink or have a light meal, if desired. The day after surgery the patient resumed a normal diet, and became fully mobilized, to be discharged home between 2 and 5 days after operation. Ultrasonography was carried out routinely before discharge to check the mesh position and to look for serohaematomas. Patients were recommended to wait until the stitches had been removed before resuming normal physical activity 8–10 days after operation.

A total of 15 surgeons took part in the operation programme; although most operations were performed by three senior surgeons in the early years, later other colleagues were introduced into the programme[9]. Trainees currently perform almost half of the operations, rising from 1·7 per cent in 1993 to 44·9 per cent in 2001. Recurrent hernia repairs were performed only by senior surgeons.

Follow-up

Upon discharge, each patient received a written schedule for follow-up examination in the hernia consultation office. Patients were re-examined 4 weeks, and 1, 2, 5 and 10 years after operation by an independent observer. Unreliable patients were contacted regularly by a special telephone service and reminded to keep their appointments. A recurrence was defined as a reducible bulge at the site of the previous repair. Follow-up ranged from 1 to 97 (median 22) months. Some 93·1 per cent of patients were examined clinically at least once after discharge; only 6·9 per cent of patients were completely lost to follow-up. Postoperative discomfort was judged by a visual analogue scale that ranged from 0 to 100.

Statistical analysis

Data were processed using medians and ranges. Fisher's exact test and the non-parametric Wilcoxon test were applied for statistical analysis according to data type. $P < 0.05$ was considered significant.

Results

Between April 1993 and December 2001, 8050 TAPP procedures were performed on 6479 patients, 5862 men (90·5 per cent) and 617 women (9·5 per cent). The median age of the patients was 59 (range 16–97) years; 1411 patients (21·8 per cent) were over 70 years, 378 (5·8 per cent) were over 80 years and 37 (0·6 per cent) were over 90 years old. The median body mass index (BMI) was 24·8 (range 14·0–39·3); 337 patients (5·2 per cent) had a BMI of more than 30.

There were 6904 primary hernias (85·8 per cent), and 1146 repairs (14·2 per cent) were done for recurrent hernia. Some 4908 patients (75·6 per cent) had unilateral hernia repair and 1571 patients (24·4 per cent) had simultaneous bilateral repair. TAPP was used for all types of groin hernia (*Table 1*), including 440 scrotal hernias (5·5 per cent). A total of 280 hernias (3·5 per cent) were irreducible; 92 (1·1 per cent) of these were acutely incarcerated and five needed simultaneous resection (ileum, two; appendix, one; omentum, two). TAPP was completed laparoscopically in 99·9 per cent of hernia repairs. A conversion was necessary in ten procedures: two for bleeding from a branch of the pubic artery, one for unclear anatomy in a scrotal hernia, one for a bleeding from the omentum and six for adhesions.

The median duration of operation was 47 (range 20–276) min for unilateral primary hernia, 45 (range 20–

Table 1 Types of hernia treated by laparoscopic transperitoneal hernioplasty

Nyhus type[10]	No. of hernias
II (indirect)	1915 (23·8)
IIIa (direct)	2625 (32·6)
IIIb (indirect, combined)	2141 (26·6)
IIIc (femoral)	223 (2·8)
IV (recurrent)	1146 (14·2)
Total	8050 (100)

Values in parentheses are percentages

Table 2 Complications following 8050 consecutive laparoscopic transperitoneal hernioplasties

	No. of repairs
Intraoperative	
Injury to lateral cutaneous nerve of thigh	26 (0·3)
Bleeding (trocar site)	14 (0·2)
Bowel injury	9 (0·1)
Bladder injury	8 (0·1)
Bleeding (inguinal)	5 (0·1)
Injury to vas deferens	2 (0·0)
Other	6 (0·1)
Postoperative	
Urinary retention	44 (0·5)
Bleeding (inguinal)	20 (0·2)
Epididymitis or orchitis	8 (0·1)
Mesh infection	8 (0·1)
Urinary tract infection	6 (0·1)
Thrombosis	5 (0·1)
Seroma (reoperation)	5 (0·1)
Allergic reaction	4 (0·0)
Wound infection	3 (0·0)
Ileus (reoperation)	3 (0·0)
Bleeding (trocar site)	3 (0·0)
Ileus (conservative)	2 (0·0)
Myocardial infarction	1 (0·0)
Stroke	1 (0·0)
Pulmonary embolism	1 (0·0)
Long term	
Trocar-site hernia	57 (0·7)
Testicular atrophy	4 (0·0)
Seroma (persistent)	3 (0·0)
Ileus (reoperation)	2 (0·0)
Chronic pain (reoperation)	2 (0·0)
Other	3 (0·0)
Total	255 (3·2)

Values in parentheses are percentages

265) min for a recurrent hernia, 65 (range 20–276) min for a scrotal hernia and 70 (range 30–300) min for bilateral hernias.

Some 255 complications were observed (morbidity rate 3·2 per cent) (*Table 2*). A single elderly man died from pulmonary embolism following repair of bilateral irreducible scrotal hernias (mortality rate 0·02 per cent). The overall morbidity and reoperation rates were similar for all types of hernia repair (*Table 3*), although the frequency of serohaematoma that needed aspiration was 2·7 per cent after unilateral primary hernia repair, 4·8 per cent after recurrent hernia repair and 4·0 per cent after bilateral repair, rising to 12·5 per cent following scrotal hernia repair ($P < 0{\cdot}001$).

The overall hernia recurrence rate was 0·7 per cent (60 of 8050). Although there was no significant difference in recurrence rate after repair of unilateral primary hernias (0·8 per cent), recurrent hernias (1·1 per cent) or bilateral hernias (0·6 per cent), that after repair of scrotal hernias was significantly higher at 2·7 per cent ($P = 0{\cdot}04$ *versus* non-scrotal repair) (*Table 3*).

The median time to return to work, including hospital stay, was 15 (range 2–100) days after all hernia repairs, 14 days after unilateral primary hernia repair, 21 days after recurrent hernia repair, 19 days after scrotal hernia repair and 15 days after bilateral hernia repair (P not significant).

After the learning phase and introduction of the above-mentioned modifications, there was a significant reduction in operation duration, and morbidity and recurrence rates. After the first 600 procedures, the median duration of operation decreased from 50 to 42 min, the morbidity rate from 9·3 to 2·6 per cent, the reoperation rate from 1·3 to 0·4 per cent and the recurrence rate from 4·8 to 0·4 per cent.

At the beginning of this experience the three senior surgeons had considerable variation in morbidity (range 5·3–11·3 per cent) and recurrence rates (2·0–6·0 per cent). After about 300 hernia repairs their results became almost identical (2·0–3·1 per cent and 0·3–0·4 per cent respectively).

Apart from a longer operating time, the results of TAPP by specialist trainees (who operate independently) and junior trainees were similar to those obtained by the three consultant surgeons beyond the learning curve (after first 300 individual procedures) (*Table 4*).

Discussion

The efficacy of a new operation can be assessed only on an unselected set of patients, and only when surgery has been performed as part of a normal hospital routine. The present results show that TAPP has a significant learning curve and therefore may carry risks for the patient. However, they also show that, as a surgeon's experience grows, TAPP can be performed increasingly quickly, safely and with a low

Table 3 Morbidity, reoperation and recurrence rate in relation to hernia type in 8050 consecutive laparoscopic transperitoneal hernioplasties

	Unilateral primary hernia	Recurrent hernia	Scrotal hernia	Bilateral hernia repair*
No. of hernia repairs	4222	1146	440	3142
Morbidity rate (%)	3·0	4·5	4·8	2·7
Reoperation rate (%)	0·4	1·0	1·1	0·5
Recurrence rate (%)	0·8	1·1	2·7	0·6

*Some 3142 hernias repaired in 1571 patients. Results refer to number of hernias repaired

Table 4 Results of laparoscopic transperitoneal hernioplasty according to grade of surgeon and experience

	Senior surgeons (*n* = 3)			
	≤ 300 individual operations	> 300 individual operations	Specialist trainees (*n* = 3) All operations	Trainees (*n* = 9) All operations
No. of hernia repairs	900	5240	1285	625
Operating time (min)*	50 (20–155)	40 (15–265)	50 (15–276)	53 (22–160)
Morbidity rate (%)	7·1	2·4	3·0	2·2
Reoperation rate (%)	1·2	0·4	0·5	0·3
Recurrence rate (%)	3·6	0·4	0·3	0·2

*Values are median (range)

recurrence rate for all hernia types, including femoral hernia. The results are better those of conventional surgery[11–16], especially for recurrent, bilateral hernia or scrotal hernias, even taking into consideration the duration of operation.

The most significant complication was intraoperative intestinal damage, which occurred in nine patients. As eight of these nine patients had undergone previous abdominal surgery and adhesions were to be expected, laparoscopic surgery should be performed in such patients only after serious scrutiny of the indication, and by an experienced surgeon. In these patients the optic trocar should be introduced under direct vision using the Hasson technique. If inspection reveals extensive adhesions, conversion to a conventional repair is recommended.

Similarly, five of the eight patients with urinary bladder damage had had previous surgery, particularly transabdominal prostatic resection, with corresponding tight adhesions. In these patients laparoscopic hernioplasty should be considered carefully. Fortunately, none of the bladder lesions had serious consequences; they were detected immediately and repaired, and/or a urinary catheter was inserted for a few days.

Of the eight patients who developed an infection in the area of the mesh site, only three required removal of the mesh. In the other five, the infection healed without complications after operative revision and a lavage–suction drain. To what extent perioperative antibiotics are useful is a subject of controversy as studies with the appropriate statistical power are lacking. Generally an infection risk of 1–2 per cent must be taken into account, even during aseptic operations[17]. The low (0·1 per cent) rate of infection here is a strong argument in favour of perioperative prophylactic antibiotics.

In spite of the high proportion of patients over 70 years of age (21·8 per cent), cardiopulmonary complications arose in only two patients. This is the result of low pain levels after laparoscopic hernioplasty that allowed good patient mobility, just a few hours after the operation.

The low rates of testicular atrophy and late postoperative chronic pain (both less than 0·1 per cent) were notable. This may be a benefit of laparoscopic hernioplasty, especially when repairing a recurrent hernia after previous conventional surgery[18] or a large scrotal hernia.

Critics of laparoscopic hernioplasty maintain that this technique is difficult to learn and can be performed only by a few experts, so may not be suitable for surgeons in training[9]. The present results show that TAPP can be standardized and performed safely by trained laparoscopic surgeons. Specialist trainees and trainees who learned laparoscopic hernia repair after technical modifications had been made were introduced to a clinical routine. First the young surgeons had to guide the camera (at least 50 times) and then they were allowed to operate on easy cases (e.g. female patients, small primary direct hernia) with supervision by one of the senior surgeons. Their learning curve was shorter, as they could avoid a lot of mistakes made by the senior surgeons when they started laparoscopic hernia repair.

The present recurrence rate of 0·4 per cent shows that TAPP may have advantages over even the best suture repairs[11] and open mesh repairs[19,20], although a final assessment is not yet possible owing to the comparatively short follow-up. After analysis of the initial results, mistakes (e.g. mesh too small, slitting the mesh and insufficient parietalization) could be recognized and eliminated[21–24], although a relatively high recurrence rate is still seen after scrotal hernia repair.

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Correspondence

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Prognostic significance of the circumferential resection margin following total mesorectal excision for rectal cancer (*Br J Surg* 2002; 89: 327–34)

Sir

The authors have excluded patients who received preoperative radiotherapy; however, 3 per cent of their cohort had adjuvant chemotherapy. Were these patients excluded along with the patients who died from a non-cancer-related cause when survival analyses were performed? Further, it is a setback to see a study with large numbers that has a median follow-up of only 29 (range 14–60) months. A 5-year minimum follow-up should be mandatory to draw any valid conclusions on metastases and death. We appreciate the authors' use of multivariate analysis to assess independent prognostic effect and would like to know whether vascular invasion was included as other studies have shown its significance on survival and metastases from colorectal cancer[1,2].

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Author's reply

Sir

As the causes of death can be difficult to identify in a large national study, the use of cancer-specific survival may be biased. The date of death, however, cannot be manipulated, and thus we preferred to use crude survival. The survival analyses did not exclude patients who had chemotherapy. Most likely the impact of an involved circumferential margin is even more evident for cancer-specific survival. It may be argued that a median follow-up of 29 months is short. Nevertheless, 161 patients were followed beyond 40 months, and the cohort was large enough to identify significant predictors for local recurrence, metastasis and death. Vascular invasion was not implemented in the multivariate analysis as this variable has not been reported routinely by the pathologists. The main message of the present study, in which 76 per cent of patients had high-risk tumours (Dukes' B and C) and there was no use of radiotherapy, is the importance of obtaining a tumour-free margin. Surgical technique does matter.

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Surgical repair of ruptured thoracic and thoracoabdominal aortic aneurysms (*Br J Surg* 2002; 89: 442–5)

Sir

The authors have produced excellent results in this difficult group of patients, but even in their hands open surgery is associated with significant morbidity and delayed mortality. There appears to be a discrepancy between the 1-year survival rate for those who had surgery quoted in the abstract (65 per cent) and the figure in the text (53 per cent). While we appreciate that this was a retrospective study, no data have been included about the number of patients turned down for surgery. The authors make no mention of the use of endovascular repair for thoracic aneurysms. At present branched stent-grafts for repair of thoracoabdominal aneurysms remain experimental. However, endoluminal repair for type B aortic dissection and descending thoracic aneurysms appears to be a viable alternative to open surgery. In addition, some type I thoracoabdominal aneurysms may be amenable to stent-graft repair.

Endoluminal repair is much less invasive and can be offered to patients who are unfit for open surgery. Since 1997 we have treated 62 patients, including 24 non-elective procedures. The indications for treatment included symptomatic degenerative aneurysms (seven), false aneurysms associated with infection (six), acute type B dissection (four), trauma (transection in three and traumatic dissection in one), Takayasu's vasculitis causing rupture of the descending thoracic aorta (one), symptomatic aneurysm associated with chronic dissection (one) and secondary aorto-oesophageal fistula (one). The 30-day survival rate was 83 per cent (20 of 24) and the survival rate at 1 year was 71 per cent (17 of 24). The complications included: paraplegia (one) which resolved completely following cerebrospinal fluid drainage, stroke (one) from which the patient made a good recovery, distal endoleak (one) requiring an extension cuff, and persistent proximal endoleak (one) necessitating removal of the graft at open repair. One of the problems in reporting results for non-elective endoluminal repair is the lack of certainty that there is 'blood outside the aorta', which can be ascertained only at open surgery. However, all patients in our series had indications for urgent surgical repair but were considered too high risk by either cardiothoracic surgeons or anaesthetists.

It is important to recognize that in certain patients endoluminal repair is a safe alternative to open surgery. The lack of durability of the stent-grafts may be the Achilles heel of endoluminal repair but the poor long-term survival of this group, as demonstrated by this paper, may mean that this has less importance.

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Randomized clinical trial of Ligasure^{TM} *versus* open haemorrhoidectomy (*Br J Surg* 2002; 89: 154–7)

Sir

The authors could not have drawn all their conclusions because of their study design. Both groups of patients underwent haemorrhoidectomy under general anaesthetic. There was no mention of which anaesthetic agents were used or whether every patient had the same anaesthetic; different anaesthetics have different analgesic properties. There was also no record of perioperative parenteral analgesic administration. Both of these can significantly affect the postoperative pain scores. The authors recorded the patients' pain scores as the pain experienced at the time of waking each day. It might have been more appropriate to score the maximum pain that day, or the pain after defaecation. The inclusion of both men and women may also have skewed the results. Men and women have different thresholds for recording pain[1] and also report the intensity of pain differently[2,3]. These factors could have affected the conclusions of an otherwise well thought out study.

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Nyhus and Condon's Hernia. 5th ed.

R. J. Fitzgibbons Jr and A. G. Greenburgh (eds)
280 × 120 mm. Pp. 650 Illustrated. 2001. Philadelphia, Pennsylvania. Lippincott Williams and Wilkins $179·00

This large book deals comprehensively with all types of abdominal hernia and comprises 50 chapters from 98 distinguished contributing authors. As in all multiauthor books, the style and organization of the content varies from chapter to chapter and there is some repetition. The aetiology, history and anatomy of groin hernias occupies the first nine chapters. Next come detailed accounts of different methods for their repair. Some repairs, such as Bassini's, are of largely historical interest and others, such as the Kugel approach, will not find many advocates. Laparoscopic repairs are comprehensively described, although few surgeons would now use the intraperitoneal on-lay mesh, which has a chapter to itself. There are five chapters about the complications of groin hernia repair, including the important problem of postoperative pain. Ventral incisional (seven chapters), paediatric and diaphragmatic hernias are considered in detail. The final part of the book includes useful but disparate chapters on anaesthesia, pain management, clinical research, defects of pelvic support in women, biomaterials and assessment of the quality of hernia repair. Some chapters are followed by germane comments from the editors. This is a reference book that will be of value to both the established surgeon and the surgical trainee.

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Fifty Cases of Peripheral Vascular Interventions

C. J. White (ed.)
240 × 160 mm. Pp 219 Illustrated. 2002. London: Martin Dunitz £34·95.

This book attempts to provide a better understanding of peripheral vascular interventions. The authors are mainly from the USA, with one contribution each from Argentina and France. Essentially there are 50 cases of 'how I did it', which are 'intended to serve as examples or templates for practitioners wishing to expand their fund of knowledge regarding peripheral vascular disease' (editor's words). Each case provides a background procedure and commentary. Only one of these commentaries (number 48) offers an alternative approach to the management of the case. More importantly, there are a number of cases for which there is no evidence base for the treatment offered. My radiological colleagues and I were surprised to see that laser-assisted angioplasty still has a place in the 21st century. Unfortunately the procedure section includes a large number of technical devices that are not available in the UK. The book therefore has little to offer British vascular surgical trainees wishing to obtain a basic grounding in vascular intervention, who would be better advised to read a more simplified text. For UK vascular surgeons and radiologists, this book offers insight into what may be possible, but there is little evidence on which to base these skilful interventions.

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Scientific Surgery

AbuRahma AF, Hannay RS, Khan JH, Robinson PA, Hudson JK, Davis EA. Prospective randomized study of carotid endarterectomy with polytetrafluoroethylene *versus* collagen-impregnated Dacron (Hemashield) patching: perioperative (30-day) results. *J Vasc Surg* 2002; **35:** 125–30.

Two hundred procedures were studied. Polytetrafluoroethylene patching significantly reduced the number of perioperative strokes (0 *versus* 7, $P = 0{\cdot}02$) and was associated with a reduced rate of carotid thrombosis (0 *versus* 5) and death (0 *versus* 2), but it took longer to achieve haemostasis after endarterectomy.

Ho Y-H, Seow-Choen F, Tan M. Colonic J-pouch function at 6 months *versus* straight coloanal anastomosis at 2 years: randomized controlled trial. *World J Surg* 2001; **25:** 876–81.

Forty-two patients were studied after ultra-low anterior resection. After 6 months patients with a pouch had less frequent defaecation and less soiling but by 2 years there were no functional differences between the two groups.

Yu W, Whang I, Chung HY, Averbach A, Sugarbaker PH. Indications for early postoperative intraperitoneal chemotherapy of advanced gastric cancer: a prospective randomized trial. *World J Surg* 2001; **25:** 985–90.

In this study, which included 248 patients, the overall survival rate was improved by chemotherapy from 38 to 54 per cent ($P = 0{\cdot}03$). The survival advantage remained significant for patients with serosal tumour invasion and lymph node metastases.

Braga M, Gianotti L, Nespoli L, Radaelli G, Valerio DC. Nutritional approach in malnourished surgical patients. A prospective randomized study. *Arch Surg* 2002; **137:** 174–80.

Some 150 patients were divided into three groups. Both preoperative and postoperative additional nutrition reduced the total number of complications ($P = 0{\cdot}02$) and shortened the duration of hospital stay by 2–3 days ($P = 0{\cdot}01$).

Spencer GM, Thorpe SM, Blackman GM, Solano J, Tobias JS, Lovat LB *et al.* Laser augmented by brachytherapy *versus* laser alone in the palliation of adenocarcinoma of the oesophagus and cardia; a randomized study. *Gut* 2002; **50:** 224–7.

There was no morbidity in the 11 patients randomized to a single dose of radiotherapy. In this small study there were no differences in clinical outcomes.

The Propranolol Aneurysm Trial Investigators. Propranolol for small abdominal aortic aneurysms: results of a randomized trial. *J Vasc Surg* 2002; **35:** 72–9.

Some 548 patients with an aorta 3–5 cm in diameter were followed for a mean of 2·5 years. The propranolol was poorly tolerated: 42 per cent stopped the active medication. Mean aneurysm growth rates were similar (0·22 cm per year *versus* 0·26 cm per year with placebo, P not significant).

Cao P, De Rango P, Zannetti S. Eversion *versus* conventional carotid endarterectomy: a systematic review. *Eur J Vasc Endovasc Surg* 2002; **23:** 195–201.

This review included 2590 procedures from five studies. There were no statistically significant differences in perioperative stroke and death rates, but eversion endarterectomy was associated with a lower rate of restenosis.

Kitano S, Shiraishi N, Fujii K, Yasuda K, Inomata M, Adachi Y. A randomized controlled trial comparing open *versus* laparoscopy-assisted distal gastrectomy for the treatment of early gastric cancer: an interim report. *Surgery* 2002; **131:** S306–11.

The specimens produced for histology by the two techniques were similar. The laparoscopically-assisted method was less painful after operation and improved early pulmonary function in this study of 28 operations.

Witte J, Goretzki PE, Dotzenrath C, Simon D, Felis P, Neubauer M *et al.* Surgery for Graves' disease: total *versus* subtotal thyroidectomy – results of a prospective randomized trial. *World J Surg* 2000; **24:** 1303–11.

The effect of three different procedures on Graves' ophthalmoplegia was studied in 150 patients. Radical subtotal resection (remnant less than 4 ml) optimized the clinical results but minimized the risks of postoperative hypocalcaemia.

Smith JJ, Brown L, Greenhalgh RM, Davies AH. Randomized trial of preoperative colour duplex marking in primary varicose vein surgery: outcome is not improved. *Eur J Vasc Endovasc Surg* 2002; **23:** 336–43.

This study included 149 consecutive patients. Preoperative duplex marking did not affect the outcome of surgery when assessed with further duplex imaging at 6 weeks and 1 year. Quality of life was improved in both groups by surgery, but there was no difference in the improvement between the groups.

Wolterbeek JH, van Leeuwen AAM, Breslau PJ. Skin closure after infrainguinal bypass surgery: a prospective randomized study. *Eur J Vasc Endovasc Surg* 2002; **23:** 321–4.

Skin closure after 170 procedures was randomly allocated to continuous polyamide sutures or staples. There was no difference in complication rates (superficial infection: 8 per cent sutures *versus* 2 per cent staples; deep infection: 1 per cent each), but wound closure was quicker with staples.

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Statistics

For detailed guidance on the handling of statistical material consult *Br J Surg* 1991; **78**: 782–4. In evaluating a manuscript the Editors and statistical referees will consider the design of the study, the presentation and analysis of data and the interpretation of results.

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Clearly describe methods used for each analysis. Methods not in common usage should be referenced. Report results of statistical tests by stating the value of the test statistic, the number of degrees of freedom and the *P* value. Actual *P* values should be reported to three decimal places, especially when the result is not significant. The results of the primary analyses should be reported using confidence intervals instead of, or in addition to, *P* values.

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Take great care in your interpretations. Do not place undue emphasis on secondary analyses.

References

Type the references with double spacing in the Vancouver style (see **Preparation of manuscripts**). Reference to abstracts and personal communications is discouraged. Reference to unpublished communications will not be accepted.

In the text, number references consecutively by superscript:^{1} or $^{1-3}$. References cited only in tables or figures should be numbered in sequence.

BJS

British Journal of SURGERY

Volume 89
Supplement 1
June 2002

Association of Surgeons of Great Britain and Ireland and Society of Academic and Research Surgery

Joint Annual Meeting
Dublin, 22–24 May 2002

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Association of Surgeons of Great Britain and Ireland

2002 MOYNIHAN TRAVELLING FELLOWHSIP
AND DINWOODIE AWARDS

The Association of Surgeons of Great Britain and Ireland represents all general surgeons throughout the United Kingdom and the Republic of Ireland. An annual Travelling Fellowship up to the value of £4,000 is available by open competition to Specialist Registrars towards the end of higher surgical training or Consultants within three years of appointment at the closing date of this advert, to enable them to broaden their education and to present and discuss their contribution to British and Irish surgery overseas. The Dinwoodie Trust will offer one or two further awards of approximately £1,500.

Candidates must be residents of the United Kingdom or the Republic of Ireland but need not be either Fellows or Affiliate Fellows of the Association; however they should be engaged in general surgery or in one of its specialties. A full Curriculum Vitae should be submitted giving details of past and present appointments and publications, together with a detailed account of the proposed programme of travel, costs involved and objectives to be achieved. Shortlisted candidates will be interviewed by the panel at the Association, at 10.30am on Thursday 24 October 2002. The panel will pay particular attention to originality, scope and feasibility of the proposed itinerary. The successful candidate will be expected to act as an ambassador for British and Irish Surgery and should be fully acquainted with the aims and objectives of the Association of Surgeons and its role in surgery. After the visit the successful candidate will be asked to address the Association at its Annual General Meeting.

Deadline for applications: **Monday 7 October 2002**
Applications (15 copies) and requests for information should be submitted to the Honorary Secretary, Association of Surgeons of Great Britain and Ireland, at the Royal College of Surgeons of England, 35/43 Lincoln's Inn Fields, London WC2A 3PE.

2002 OVERSEAS SURGICAL FELLOWSHIP

The Association of Surgeons of Great Britain and Ireland, together with the **British Journal of Surgery Society**, are offering £10,000 per annum to sponsor surgeons wishing to work on a short term basis, primarily in the Third World. This Scheme is run in conjunction with the Tropical Health and Education Trust to provide support for overseas medical schools in the development of their undergraduate and postgraduate training programmes and also for research, thereby, establishing links with these centres.

Only Fellows including Full, Senior, Associate and Affiliates of the Association of Surgeons may apply and if successful, a grant of up to £2,000 will be made available to individual applicants.

Deadline for applications: **Monday 7 October 2002**

Further details are available from Mrs Nechama Lewis, The Association of Surgeons of Great Britain & Ireland, at the Royal College of Surgeons, 35/43 Lincoln's Inn Fields, London WC2A 3PE Tel: 020 7973 0300 Fax: 020 7430 9235.

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Association of Surgeons of Great Britain and Ireland and Society of Academic and Research Surgeons

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The combined meeting of these societies occurs in Dublin this year and the *British Journal of Surgery* is pleased to continue its association with these two of its affiliated societies in the production of this abstract supplement.

The Editors of *BJS* wish all participants an enjoyable and fruitful meeting in Cardiff.

The Editors
BJS

ASGBI: Breast 01–06

Wednesday, 22 May 08.45–10 00

Breast 01

Intraoperative evaluation of axillary sentinel lymph nodes using touch imprints and immunohistochemistry. Part II. Preliminary results

A.A. Salem, A.G. Douglas-Jones, H.M. Sweetland, R.G. Newcombe and R.E. Mansel

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Aims: The success of the 'Sentinel Node Biopsy' (SNB) in determining the axillary lymph node status necessitates an accurate and rapid method for intraoperative examination of the nodes in order to selectively operate on positive axillae during the same operative session. The aim of the study was to determine the feasibility and accuracy of IHC stained touch imprints in detecting axillary nodal metastases intraoperatively. Three hundred and forty-four axillary nodes from 30 patients [23 axillary node clearance (ANC), 2 axillary node sampling (ANS) and 5 SNB], were bisected, imprinted and stained with IHC. Results were compared with those of H&E sections of the same nodes.

Results: Using IHC, 29 nodes from 9 patients were positive for metastases. Using H&E, 28 nodes from 8 patients were positive, resulting in 30 per cent and 27 per cent positive cases and 8.4 per cent and 8.1 per cent positive nodes for IHC and H&E, respectively. On a patient basis, sensitivity was 100 per cent and 89 per cent, and negative predictive value (NPV) was 100 per cent and 95.5 per cent for IHC and H&E, respectively. On a node basis, sensitivity was 96.7 per cent and 93.3 per cent and NPV was 99.6 per cent and 99.5 per cent for IHC and H&E, respectively. Because of the absence of false positives, specificity and positive predictive value (PPV) were 100 per cent for both IHC and H&E. With ANS and SNB procedures, results were obtained within 35–50 min depending on the number of the nodes given.

Conclusion: These results indicate that IHC stained touch imprints can provide a fast and sensitive method for detecting metastases in axillary nodes during breast cancer surgery.

Breast 02

Pre-operative evaluation of radial scars (RS) and complex sclerosing lesions (CSL): are fine needle aspiration cytology (FNAC) and core biopsy (CB) useful?

D.C. Windmill, C. Holgate, P.A. Jones, J.R. Steel and R.M. Watkins

Primrose Breast Care Centre, Derriford Hospital, Plymouth, PL6 8DH

Aims: Many screen-detected RSs and CSLs are associated with malignant disease and the diagnostic accuracy of FNAC and CB in preoperative evaluation remains uncertain. Our aim was to determine the role of FNAC and CB in the management of these lesions.

Methods. The results of FNAC and CB in 54 women (age range: 36–72 years, mean 53 years) with a definitive diagnosis of RS or CSL at excision biopsy between January 1999 and January 2002 were reviewed.

Results: FNAC in 37 patients was inadequate in 15 (41%). Sixteen patients were classified as C2 (43%), three as C3 (8%), one as C4 (3%) and two as C5 (5%). CBs in 37 patients were graded as B1 in four patients (11%), B2 in 27 (73%), B3 in four (11%), B4 in two (5%). Four cases of ductal carcinoma in-situ and three of invasive carcinoma were identified following excision biopsy. None was diagnosed pre-operatively by CB. One invasive carcinoma was diagnosed on FNAC. One patient had a false positive result on FNAC. Only 14 of 37 women (39%) had positive diagnosis of RS or CSL on pre-operative CB. A further six women (16%) had CB results suggestive of RS or CSL.

Conclusion: FNAC and CB are insufficient to accurately diagnose pathology associated with RS or CSL. Excision biopsy remains essential.

Breast 03

Tubular carcinoma of the breast: is long-term survival assured?

N. Beechey-Newman, A. Bashorun, K. Ryder and A.E. Young

Breast Unit, Guy's & St Thomas' Hospitals, London, UK

Aims: To review the long-term (20–25 years) survival of patients with pure tubular carcinoma and to investigate whether this lesion does confer a better survival compared with the control group of other commoner grade I invasive ductal carcinomas of no specified type (NST). Case records from 1975 to 2000 were obtained for all grade I invasive ductal carcinomas (NST and TC) treated at Guy's Hospital, London. There were 73 patients with pure TC and 420 grade I (NST). The mean follow-up was 9.7 years. All patients had tumourectomy and complete axillary dissection. Kaplan–Meier survival curves were compiled and statistical analysis was carried out using the log rank method.

Results: There was no correlation between histological type and age ($P = 0.102$) or nodal status ($P = 0.205$). In the TC group survival was significantly better in patients without lymph node metastasis ($P = 0.0412$). No significant statistical differences in long-term survival of patients with TC and those with G1 (NST) were identified ($P = 0.487$). Moreover the similarity in overall survival remained after subdivision into node negative ($P = 0.23$) and node positive ($P = 0.70$) groups.

Conclusion: Although pure tubular carcinoma has been thought to be a favourable subtype of breast cancer, it is concluded that its overall long-term survival is no better than that of the commoner grade I invasive carcinoma of nonspecific type. Therefore tubular carcinoma should continue to be regarded as potentially life-threatening in the longer term and accordingly should not merit an attenuated treatment or follow-up protocol.

Breast 04

Role of a polymorphism in 3′-untranslated region of vascular endothelial growth factor gene in breast cancer

S.P. Balasubramanian, N.J. Brown and M.W. Reed

Department of Surgery and Surgical Oncology, University of Sheffield, Sheffield, UK

Aims: Vascular endothelial growth factor (VEGF) is over expressed in breast cancer and correlates with tumour progression and prognosis. Many polymorphisms occur in the VEGF gene including a single nucleotide polymorphism in the 3′-untranslated region (936 C>T), which is significantly associated with plasma VEGF levels. We aimed to study the role of this polymorphism in breast cancer susceptibility and severity.

Methods Blood samples were collected from 1575 breast cancer patients (attending the follow-up clinic at the Royal Hallamshire Hospital, Sheffield) and controls (attending the Breast Screening service in Sheffield). DNA extraction, PCR amplification of the target sequence and genotyping for the 936 C > T polymorphism using the ABI prism 7200 Sequence Detection System (Taqman™ allelic discrimination method) was performed (South Sheffield Research Ethics Committee, Ref. No. SS98/137).

Results: The genotype frequencies of the polymorphism in the control population [75 per cent, 2 per cent and 23 per cent for the 1·1, 2·2 and 1·2 genotypes, respectively] are in Hardy–Weinberg equilibrium. Overall, there is no association between the polymorphism and breast cancer [Odds ratio = 1.05, with 95 per cent confidence intervals of 0.83 and 1.33]. Analysis of the first 1284 patients and controls suggested an interaction between hormone replacement therapy and the genotype. This was not confirmed in the entire dataset of 1575 individuals. There is no association of the genotype with tumour grade, nodal status and vascular invasion.

Conclusion: The 936 > T polymorphism in the 3′UTR of the VEGF gene is not associated with breast cancer susceptibility and/or severity.

Breast 05

The association of ghrelin with tumourigenic genes in normal and malignant breast tissue

C. Laban, W. Ugunkolade, S. Bustin, P.J. Jenkins and R. Carpenter

Department of Breast Surgery, St Bartholomews Hospital, London, UK

Aims: (i) To quantify the mRNA levels of GHRH, ghrelin and their receptors in malignant and adjacent normal breast tissue, and (ii) to determine any correlation between such expression and that of the tumour associated genes, *PCNA*, c-*myc*, *Cox-2* and *VEGF 189*.

Methods: Total RNA was extracted from 60 samples of paired normal breast epithelium and carcinoma. mRNA levels of each gene were quantified using a real time, hydrolysis probe-dependent RT-PCR assay ('Taqman'), and expressed as log copy no./μg total RNA.

Results: Expression of GHRH, GHRH-R and GHS-R 1a was absent in all specimens of normal and malignant breast tissue. Ghrelin mRNA was expressed in all samples of normal tissue and cancers, but there was no significant difference between the two (median level: 1.6×10^5 *versus* 2.1×10^5 copy no./μg total RNA, respectively). In normal breast tissue there was a significant correlation between ghrelin mRNA levels and those of all genes studied. Weaker correlations were present in the cancers.

	c-*myc*	*Cox-2*	*VEGF-189*	*PCNA*
Normal tissue	0.40*	0.34*	0.41*	0.66***
Malignant tissue	0.56**	0.33*	0.37*	0.15

Correlation coefficients between mRNA copy no. of ghrelin and c-*myc*, *Cox-2*, *VEGF- 189* and *PCNA*. $^*P < 0.05$; $^{**}P < 0.0005$; $^{***}P < 0.000005$

Conclusion: The universal expression of ghrelin and its association with that of genes implicated in breast tumourigenesis suggests a role for ghrelin in both the maintenance of normal breast tissue and tumourigenesis. The absence of the GHS-R 1a strongly implies that the effects of ghrelin are mediated via a novel receptor. Whether these effects are independent of the GH/IGF-I axis remain to be determined.

Breast 06

Use of standard referral forms does not reduce inappropriate referrals to specialist breast clinics

I. Reid

Department of Surgery, Victoria Infirmary, Langside Road, Glasgow and University of Glasgow

Aims: Specialist Breast Clinics suffer from large numbers of inappropriate referrals, which prolongs waiting times for urgent cases. This study attempted to reduce inappropriate referrals by the introduction of a standardized referral performa based on NHS referral guidelines.

Methods: A previous prospective audit of new-patient referrals from primary care to the Specialist Breast Clinic showed 28 per cent of all referrals to be inappropriate according to NHS guidelines. We asked general practitioners referring patients to this breast clinic to complete a postal questionnaire regarding their knowledge of and adherence to published referral guidelines. GPs then received a copy of the NHS guidelines for referral to specialist breast clinics, and a draft referral form for breast clinic referrals. All breast clinic referrals were audited over the subsequent 3 months.

Results: Thirty-seven per cent of 225 responding GPs said they did not implement published referral guidelines. Of 309 new referrals, 80 referrals (26 per cent) were inappropriate according to referral guidelines. Ninety-three referrals (31 per cent) were designated urgent, of which 41 (44 per cent) were appropriate for urgent appointments. Nineteen patients (6 per cent of referrals) had a final diagnosis of breast cancer; 6 (32 per cent) had not been referred urgently. Thirty-one per cent of referrals were made on the new forms, and 22 per cent of referrals on these forms were inappropriate compared to 28 per cent of referrals made without the forms ($P = 0.38$, χ^2 test). The introduction of standardized referral forms based on NHS referral guidelines resulted in a poor uptake of these forms by GPs, and did not reduce the number of inappropriate referrals to this clinic.

ASGBI: General 01–06

Wednesday, 22 May 08.45–10.00

General 01

Laparoscopic cholecystectomy in patients over 80 years old – 10-year review

A L Tambyraja, S Kumar and S J. Nixon

Department of General Surgery, Western General Hospital, Edinburgh EH4 2XU, UK

Aim: To assess the mortality and morbidity of laparoscopic cholecystectomy (LC) in patients over 80 years old.

Methods: All patients aged 80 years or over who had LC in one surgical unit between January 1991 and December 2000 were reviewed. Data were collected from hospital case notes and the Lothian Surgical Audit Database as required. A record of patients' demographic details, presentation on admission, operative details, postoperative complications, duration of hospital stay, in-hospital morbidity and 30-day mortality was made.

Results: A total of 117 patients, 79 (68 per cent) women and 38 (32 per cent) men, median age 83 years (range: 80–93) had LC over the 10-year period. Main indications for LC were: chronic cholecystitis in 61 (52 per cent), acute cholecystitis in 29 (25 per cent) and gall stone pancreatitis in 11 (9 per cent). Forty-one (35 per cent) patients had pre- or postoperative ERCP. Of these 41 patients, 21 (51 per cent) required intervention for CBD stones, six (5 per cent) patients were converted to an open procedure, and 45 (38 per cent) patients had emergency LC. Postoperative complications developed in 26 (22 per cent) patients – respiratory in 10 (38 per cent), urinary in seven (27 per cent), cardiac in four (5 per cent) and intraperitoneal collections in four (5 per cent). Median postoperative hospital stay was 3 (1–31) days. Thirty-day mortality was 1 (0.9 per cent) in the series.

Conclusions: Laparoscopic cholecystectomy was performed in 117 patients over 80 years old with a mortality of 0.9 per cent. Postoperative complications, mostly cardio-respiratory, developed in 22 per cent.

General 02

Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in surgical patients on admission to a Welsh hospital

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Departments of Surgery and Microbiology, Nevill Hall Hospital, Abergavenny, UK

Aims: MRSA-induced infections in surgical patients are increasing and often have disastrous consequences. The prevalence and risk factors of MRSA colonization were studied in surgical patients.

Methods: All patients admitted to general surgical and orthopaedic wards in a Welsh District General Hospital were studied prospectively over a period of 3 months. At the time of admission, a detailed questionnaire was completed. Nasal swabs and swabs from any pre-existing wound sites in each patient were sent for culture.

Results: A total of 430 patients were studied of which 23 patients (5.3 per cent) were positive for MRSA in the initial swab. Fifteen (65 per cent) of the MRSA-positive patients were nasal carriers and the rest had MRSA colonization elsewhere in the body. Four risk factors were identified. Previous admission to nursing home was a definite risk factor when considered alone and in combination with other risk factors ($P = 0.000$). Age above 70, in univariate analysis, was a definite risk factor ($P = 0.002$), but lost significance in multivariate analysis. Male sex ($P = 0.054$) and previous hospital admission ($P = 0.051$), which were marginal risk factors when considered alone, were strengthened in multivariate analysis ($P = 0.026$ and $P = 0.007$, respectively).

Conclusion: We suggest patients with these risk factors should be screened for MRSA colonization prior to any elective surgical procedure and appropriate prophylaxis given. Preoperative prophylaxis should be considered for all high-risk patients undergoing emergency surgery.

General 03

Bacterial translocation across intestinal epithelial monolayers is a species- and strain-specific polarized process

E C Clark, P Chadwick, G. Warhurst and G L Carlson

Departments of Surgery, Microbiology and Gut Barrier Group, Hope Hospital, Salford, UK

Aims: In critical illness, bacteria may translocate across the gut epithelium, it is unclear whether all bacteria can translocate to the same extent. We hypothesized that specific bacteria–enterocyte interactions determine whether bacterial translocation occurs.

Methods: Caco-2 intestinal cell monolayers were cultured for 18–21 days in bicameral (Transwell) systems. Bacterial translocation across monolayers was studied under control and stressed (glutamine deprivation and 20 ng mL^{-1} basal tumour necrosis factor-α incubation for 6 h) conditions. Rates of apical to basal ($A^{\oplus} \rightarrow B$) and basal to apical ($B^{\oplus} \rightarrow A$) translocation were measured by inoculating apical or basal chambers with 10^5 cfu of: *Escherichia coli* (strains C25 and B2), *Pseudomonas aeruginosa*, *Streptococcus faecalis*, and *Staphylococcus epidermidis*. Translocation of bacteria was measured after 4 h by serial dilution culture of the other chamber. Results (mean ± SD; $n = 3$) were analysed by one-way ANOVA.

Results: Under control conditions, all bacteria translocated to the same extent. Polarized translocation was noted for *E. coli* C25 alone, with flux from $B^{\oplus} \rightarrow A$ almost 10-fold higher than $A^{\oplus} \rightarrow B$. In stressed monolayers, translocation increased only for *E. coli* C25.

	[Bacteria] (log cfu mL^{-1})			
Bacterial species	$A^{\oplus} \rightarrow B$ Control	$B^{\oplus} \rightarrow A$ Control	$A^{\oplus} \rightarrow B$ Stressed	$B^{\oplus} \rightarrow A$ Stressed
E. coli C25	3.3 ± 0.1	4.2 ± 0.1**	5.5 ± 0.2**	5.4 ± 0.1**
E. coli B2	3.5 ± 0.3	3.6 ± 0.2	3.6 ± 0.3	3.8 ± 0.4
P. aeruginosa	3.1 ± 0.2	3.2 ± 0.2	3.5 ± 0.3	3.4 ± 0.1
S. faecalis	3.3 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.3
S. epidermidis	3.7 ± 0.4	3.5 ± 0.2	3.3 ± 0.2	3.2 ± 0.2

**$P < 0.01$ *versus* control.

Conclusions: Polarized, stress-induced translocation is a bacterial-specific process, implying that molecular bacterial–enterocyte interactions play a mechanistic role.

General 04

Technical complications of feeding jejunostomy – a retrospective study

R S Date, W.D.B Clements and R. Gilliland

Department of Surgery, Royal Victoria Hospital, Belfast, Northern Ireland, UK

Aims: Intraoperative placement of feeding jejunostomy is a well-established method of providing access to enteral feeding. A retrospective study was undertaken to look at the technical complications related to the procedure.

Methods: Patients undergoing feeding jejunostomy in the thoracic and upper gastrointestinal surgical units at the Royal Victoria Hospital from January 1998 to December 1998 were included in the study. The charts of all the patients were studied retrospectively.

Results: Forty-two patients underwent a feeding jejunostomy in the time period studied. Thirty-one patients had a jejunostomy as an adjunct to major oesophageal or gastric surgery. In five patients, feeding jejunostomy was performed in conjunction with other palliative procedures and in six patients it was performed as a sole procedure. Nine (21.4 per cent) patients had procedure-related complications. Of these, seven (16.7 per cent) were minor including dislodgement of the tube ($n = 4$), blocked tube ($n = 2$), and intraoperative catheter damage ($n = 1$). Two (4.7 per cent) patients had major complications requiring emergency laparotomy. A single patient died as a result of a procedure-related complication.

Conclusion: Feeding jejunostomy should not be considered as a benign surgical procedure. Proper attention to surgical technique and postoperative management is essential to prevent serious or life-threatening complications.

General 05

Implementation of NCEPOD guidelines to defer urgent surgery to daytime lists may increase risk of mortality

G.N.O. Dair and D.J. Leaper

Professorial Unit of Surgery, University of North Tees, Stockton on Tees, Cleveland TS19 8PE, UK

Aims: The 1987 NCEPOD report concluded that preoperative resuscitation was sometimes compromised by undue haste to operate. This study has examined the resultant policy of not operating at night and whether there is any evidence to suggest if this delay has an effect on outcome.

Methods: Sequential physiology scores were recorded in 68 patients presenting with abdominal pathologies that required urgent surgery. The physiology components of the POSSUM, APACHE II and III and SAPS II were scored at presentation, during resuscitation and immediately before surgery.

Results: There were 57 survivors and 11 nonsurvivors. All scoring systems showed an improvement in scores during resuscitation but subsequent deterioration prior to surgery. A subset analysis of the perforations ($n = 43$; 37 survivors, six nonsurvivors) was undertaken. The age and resuscitation time was 76 (55–91) years and 25.5 (11–216) h, median and range, in nonsurvivors; and 66 (17–89) years and 11 (3–224) h in survivors; $P < 0.05$ (Mann–Whitney U-test). APACHE III scores differentiated between the survivors and nonsurvivors better than the other systems. The scores at admission, the lowest during resuscitation and before surgery were 26 (6–63), 22 (5–63) and 28 (8–72) for the survivors, respectively, and 43 (29–55), 34 (24–50) and 51 (39–58) for the nonsurvivors; $P < 0.01$ (Mann–Whitney U-test).

Conclusions: Patients undergoing urgent surgery can deteriorate during resuscitation, those over 65 years old with perforations resuscitated over 12 h are at most risk. In contradiction to the NCEPOD findings, some patients may require more expedient surgery and this has significant repercussions for the structure and organization of emergency surgical services.

General 06

Outcome of patients with severe chronic pain following repair of a groin hernia – a population-based study

B. Page, C.A. Courtney, K. Duffy, M.G. Serpell and P.J. O'Dwyer

University Departments of Surgery and Anaesthesia, Western Infirmary, Glasgow, UK

Aims: Chronic pain is the most serious long-term complication that can occur after repair of a groin hernia. The aim of this study was to assess the outcome of patients who reported severe or very severe pain 3 months after groin hernia repair.

Methods: A population-based study of all patients who underwent repair of a groin hernia between April 1998 and March 1999 in Scotland. All received a postal questionnaire 3 months after hernia repair and again 2.5 years later for those that reported severe or very severe pain at the operation site.

Results: A total of 5506 patients underwent repair of a groin hernia, 4062 (74 per cent) returned the first questionnaire, while 125 (3 per cent) reported severe or very severe pain. A total of 86 (72 per cent) of the 120 patients (five had died or could not be contacted at the original address) replied to the second questionnaire. Sixty-one (71 per cent) still reported pain which was severe or very severe pain in 22 (25 per cent) and was mild or very mild in 39 (45 per cent). Twenty-nine (47.5 per cent) sought further medical help from either their GP or surgeon. Nine (15 per cent) attended a pain clinic, while five (8 per cent) had further surgery. Chronic pain had significant effects ($P < 0.0001$) on all daily activities, including walking, work, sleep, relationships with other people, mood and general enjoyment of life.

Conclusions: Chronic pain persists in most patients who report severe or very severe pain at 3 months after hernia repair and has a significant effect on the patients' daily activities and quality of life.

ASGBI: Colorectal 01–06

Wednesday, 22 May 08.45–10.00

Colorectal 01

The 2-week wait for patients with suspected colorectal cancer: the role of fast-track barium enema

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Aim: Specific referral guidelines have been drawn up for patients with suspected colorectal cancer, requiring an outpatient appointment within 2 weeks. To meet this increased demand we evaluated the efficacy of fast-track barium enema in the management of such patients.

Methods: Patients with suspected colorectal cancer had a barium enema within 2 weeks which was double reported by two consultant radiologists. Patients with a malignancy were booked for a staging CT scan and an outpatient appointment to see a consultant colorectal surgeon. A letter with the result was faxed to the GP the same day. Patients without malignancy were referred back to the GP.

Results: Prospective data were obtained on 309 successive patients over a 16-month period. The mean time from referral was 11.5 days and 277 patients (89.6 per cent) were seen within 2 weeks. Thirty-eight patients (12.3 per cent) had a colorectal cancer. Thirty-six patients went on to have a CT scan within a mean of 19.2 days. Mean time to first clinic appointment was 36.0 days. Referrals increased over each 4-month period (66, 60, 71 and 112) but the percentage of positive barium enemas remained similar (15.2 per cent, 10.0 per cent, 12.7 per cent and 11.6 per cent). To date there has been one false-negative examination. Radiologically benign polyps alone were shown in 29 patients (10.7 per cent).

Conclusions: The provision of specialist radiology as the first intervention has enabled accurate diagnosis and rapid staging of patients with suspected colorectal cancer. In addition fast-track barium enema has reduced the impact of the 2-week wait on the outpatient management of benign colorectal conditions.

Colorectal 02

Prospective randomized clinical trial of Ligasure *versus* open haemorrhoidectomy

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Aims: This study was designed to assess a new technique for haemorrhoidectomy using the bloodless bipolar diathermy apparatus 'Ligasure'. Our aim was to establish if it had advantages over conventional open haemorrhoidectomy in terms of operating time and postoperative pain.

Methods: Thirty-four inpatients were randomized to undergo either Ligasure (18) or diathermy (16) haemorrhoidectomy. The operating time, amount of pain and postoperative analgesic requirement, postoperative complications and overall patient satisfaction were documented.

Results: The median duration of surgery was shorter in the Ligasure haemorrhoidectomy group (5.1 *versus* 9.2 min) ($P < 0.001$). There was no statistically significant difference in the postoperative pain score, but the median analgesic requirement was lower in the Ligasure group (850 *versus* 1600 mg) ($P = 0.013$). Patient satisfaction was similar for both Ligasure and diathermy haemorroidectomy.

Conclusions: Ligasure haemorrhoidectomy is a simple and bloodless technique, which is as painful as diathermy haemorrhoidectomy but associated with reduced analgesic requirement and shorter operating time.

Colorectal 03

The uses of GIA staplers in closing blind intestinal ends: the threshold intraluminal pressure causing leak and methods to counteract it

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Aims: This study assesses the intraluminal pressure required to disrupt a stapled anastamosis constructed using linear and transverse staplers. Secondly, the effect of a reinforcing sutured layer on the disruption pressure is examined.

Method: Five-centimetre segments of intestine were harvested from patients undergoing intestinal resection for malignancy ($n = 19$). Both ends were closed with a linear stapler ($n = 13$), one end was reinforced with four interrupted sutures (3/0 Poliglecaprone 25). A biluminal catheter was introduced into the lumen and fixed with a purse-string suture. Normal saline (0.9 per cent) was infused into the lumen (60 mL/min) and the bursting pressure recorded for the linear stapler, transverse stapler ($n = 6$) and reinforced stapled anastamoses ($n = 13$).

Results are expressed as a median (interquartile range) and comparison made using ANOVA with $P < 0.05$ significant.

Results: Staple lines produced using a transverse stapler disrupted at higher pressures than those produced using linear staplers, 109 (95–130) cm H_2O *versus* 55.5 (46–82) cm H_2O, $P < 0.0001$. Suture reinforced linear stapler lines disrupted at pressures twice as high as unsupported linear stapler lines, 126 (112–151) cm H_2O *versus* 72 (58.5–93) cm H_2O, $P < 0.0001$. Reinforcing a linear staple line with interrupted sutures increases the disruption pressure to a level compatible with a transverse stapler, 126 (112–151) cm H_2O *versus* 109 (95–130) cm H_2O, $P < 0.1$.

Conclusions: Different methods of stapling result in significantly different physical properties of an anastamosis. Augmentation of a staple line using interrupted sutures enhances the mechanical strength of an anastamosis.

Colorectal 04

Laparoscopic resection for colorectal cancer does not affect cancer specific outcomes: report from a case control study of laparoscopic right hemicolectomy *versus* open right hemicolectomy

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Aim: The aim of our study was to examine all the laparoscopic right hemicolectomies carried out for cancer in our unit and to compare them with a case control series of open right hemicolectomies, with a particular emphasis on long-term survival.

Methods: A retrospective case control series of right hemicolectomies comparing those done laparoscopically to an age and stage matched series of patients who underwent open surgery. Analysis was done on an intention to treat basis. Survival was analysed using the techniques of Kaplan–Meier.

Results: Ninety-nine patients were included, 33 laparoscopic, 66 open (mean age 69.7 years). The distribution of Dukes stage was the same between the two groups and mean potential follow-up period was 65.7 months. There were 6 laparoscopic conversions. Days nil by mouth were significantly shorter in the laparoscopic cohort, mean of 2.4 days compared with a mean of 3.65 days ($P = 0.005$ Mann–Whitney). Days the patients required parenteral opiates were significantly shorter in the laparoscopic cohort, mean number of days 2.5 compared with 4.5 days in the open group, ($P = 0.008$ Mann–Whitney). Overall survival was compared between the open and laparoscopic groups; there was no difference seen, with a mean overall survival of 40 months in the laparoscopic cohort compared with 39.4 months in the open cohort ($P = 0.348$ log rank).

Conclusion: Laparoscopic right hemicolectomy for cancer does not compromise long-term survival and affords the advantage of a shorter period of postoperative ileus and decreased analgesia requirements.

Colorectal 05

Determining the risk of perioperative death in colorectal cancer surgery

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Aims: The purpose of the study was to establish a simple method for the identification of those patients who are at risk of perioperative death in colorectal cancer surgery.

Methods: The value of POSSUM physiological scores and ASA grades in the prediction of perioperative death was determined in a prospective study of 661 patients undergoing colorectal cancer surgery. ASA grades were recorded by anaesthetists in 647 patients and POSSUM physiological scores were calculated by surgeons for all patients.

Results: The overall perioperative mortality was 2 per cent (2 per cent in elective operations and 4 per cent after emergency surgery). In the prediction of perioperative death, a POSSUM physiological score >30 had a sensitivity of 10 per cent, a specificity of 98.2 per cent and a positive predictive value of 26.2 per cent. By comparison, ASA grade 4 had a sensitivity of 27.6 per cent, a specificity of 98.9 per cent and a positive predictive value of 53.3 per cent. The death rates in ASA grade 4 patients were 18.2 per cent in patients aged <80 years and 50 per cent in those aged >80 years. Patients who were ASA grades 1, 2 or 3 had mortality rates of 0, 1.2 per cent and 2.4 per cent, respectively.

Conclusions: Respectable overall mortality rates in large series of patients conceal the risks of death in patients with serious comorbidities. ASA grades have significant value in identifying patients who are at risk of perioperative death in colorectal cancer surgery and the level of risk can be identified in individual patients.

Colorectal 06

Quality of life and level of pain in a randomized controlled trial between accelerated and traditional care pathways after major colorectal surgery

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Aims: Fast track postoperative protocols can yield a 4.3-day (d) stay after intestinal surgery, compared to 7–10 d with traditional (TRAD) approaches. Patient satisfaction, quality of life and pain are poorly understood after abdominal surgery, and have not been compared after fast track and traditional care.

Methods: Sixty-four intestinal resection cases were randomly allocated to CREAD (controlled rehabilitation with early ambulation and diet) or TRAD. CREAD cases received: no NG tubes; ambulation and liquids on d 1; soft diet, oral analgesia on d 2. TRAD patients had: NG tubes; liquids, oral analgesia and diet after bowel function; ambulated on d 2. Postoperative endpoints at discharge, d 10 and d 30 used the Short Form-36 quality of life form, Cleveland clinic global quality of life scale (CGQL) and the McGill pain score.

Results: Length of stay including readmissions was 5.4 d in CREAD and 7.1 d in TRAD patients ($P = 0.02$). Changes in the mental component of the SF36 (reduced from 51 to 42, $P < 0.01$), and McGill score (increased from 3.9 to 7.6, $P < 0.05$) in CREAD patients at discharge, were resolved at d 10 and d 30, and are attributed to the shorter stay of CREAD patients. There were no differences in any other variable at any time.

Conclusions: Patients using the CREAD had a shorter postoperative stay, without altered quality of life or pain scores on d 10 and d 30 after surgery, when compared to patients managed by the TRAD approach. It should be considered as a primary care pathway for patients undergoing intestinal resection.

ASGBI: Transplantation 01–06

Wednesday, 22 May 08.45–10.00

Transplant 01

Warm *ex-vivo* kidney perfusion permits pretransplant evaluation of renal function

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Aims: Renal preservation by normothermic machine preservation of kidneys may permit pretransplant measurement of organ function, and therefore viability, and may offer the prospect of improved organ preservation. The aim of this study was to compare the efficacy of preservation by normothermic *ex-vivo* perfusion with conventional cold techniques.

Methods: Groups of pigs ($n = 5$) underwent left nephrectomy following warm ischaemic times (WIT) of 0 or 30 min. Kidneys were preserved either by static cold storage (UW solution, 4 °C) (CS), cold machine perfusion (UW solution 3–8 °C, 60 mmHg systolic) (CP) or warm machine perfusion (WP) using a tissue culture fluid and perfluorocarbon emulsion to increase oxygen capacity. *Ex-vivo* renal function was measured during WP by creatinine clearance. After 24 h, the kidney was auto-transplanted and a right nephrectomy performed.

Results: *Ex-vivo* creatinine clearance was higher in the 0 WIT group (6.6 ± 5.3 *versus* 1.3 ± 1.4 mL min^{-1}, $P = 0.01$). Fourteen-day animal survival rates following transplantation of 0 WIT kidneys preserved by CS, CP and WP were 3/5, 4/5 and 4/5, respectively (N.S.). Mean (SD) area under the creatinine curve levels following CS, CP and WP were 11 062 (6117), 9247 (8493) and 11 458 (5877), respectively (N.S.). Only 1/5 animals in each group survived for 14 days after a 30-min renal WIT.

Conclusions: This study demonstrates that warm perfusion using a perfluorocarbon-based oxygen carrying is associated with equivalent efficacy for renal preservation as conventional hypothermic techniques. A further finding good *ex-vivo* function is associated with high rates of post-transplant renal viability.

Transplant 02

How great is the survival advantage of transplantation over dialysis in elderly patients?

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Aim: Patients over 60 years represent 66 per cent of all new patients starting dialysis in Scotland. This study investigates whether or not transplantation provides a survival benefit compared with dialysis in elderly patients.

Methods: A total of 325 patients over 60 years were listed for transplantation between 1 January 1989 and 31 December 1999 and followed up until 31 December 2000. Socio-demographic, listing, transplant and comorbidity data were obtained from the national renal and transplant databases and case-notes review. Survival was compared between those who were transplanted and those who remained on dialysis by the end of the follow-up period.

Results: A total of 128 patients (39.4 per cent) received first transplant and 197 patients (60.6 per cent) remained on dialysis. The patients who were transplanted were younger at listing ($P < 0.0001$), lived nearer the transplant centre ($P = 0.043$), spent less time on the active waiting list ($P < 0.0001$) and had less ischaemic heart disease ($P = 0.024$), cerebrovascular disease ($P = 0.03$) and arrhythmias ($P = 0.016$) compared with those who remained on dialysis. The mortality rates were 0.16 and 0.10 per patient-year for dialysis and transplant, respectively. A Cox proportional hazard model adjusted for socio-demographic and comorbidity factors found that patients who remained on dialysis had a significantly increased risk of death compared with transplant recipients (relative risk 0.30; 95 per cent CI 0.13–0.70, $P < 0.0001$). The adjusted estimated 1, 5 and 10 years survival rates for the two groups are shown in the table ($P < 0.0001$, log rank).

Survival (years)	Remain on dialysis $n = 197$ (%)	Transplanted $n = 128$ (%)
1	94	98
5	56	82
10	0	23

Conclusions: Renal transplantation offers a significant survival advantage over dialysis in elderly patients with end-stage renal failure who are considered suitable for transplantation.

Transplant 03

De novo tacrolimus-based immunosuppression reduces renal allograft fibrosis compared to neoral: a prospective randomized clinical trial

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Aims: The aim of this study was to compare the effect of neoral cyclosporine and tacrolimus-based therapy on the development of renal allograft fibrosis (chronic allograft nephropathy, CAN) in a prospective randomized trial.

Methods: A total of 102 renal transplant patients were randomized to either microemulsion cyclosporine (neoral) or tacrolimus-based immunosuppression. Renal transplant interstitial fibrosis was quantified using computerized histomorphometric measurement of picrosirius red-stained 1-year protocol renal transplant biopsies, and levels of interstitial fibrosis were compared in relation to observed efficacy and toxicity profiles of the two drugs.

Results: There was a significant increase in allograft interstitial fibrosis in the neoral treated (sirius red area fraction staining 24.9 ± 11.1) compared to the tacrolimus-treated patients (15.9 ± 6.5, $P = 0.002$, Student's *t*-test). There was no significant difference in the demographic characteristics between the patient groups or in the incidence of acute (35 per cent tacrolimus *versus* 36 per cent neoral, $P = 0.8$, Student's *t*-test) or steroid resistant rejection (tacrolimus and neoral 10 per cent, $P = 0.9$, Student's *t*-test). There was a statistical trend ($P = 0.07$, Chi-square) towards a higher incidence of insulin resistance in the tacrolimus group (post-transplant diabetes mellitus, glucose-tolerance testing). Neoral was associated with a significant increase in total cholesterol (MANOVA, $P = 0.03$) and LDL levels (MANOVA, $P = 0.02$).

Conclusion: These data suggest that neoral-based therapy promotes increased interstitial allograft fibrosis compared to tacrolimus in renal transplants in association with a poorer safety profile and equivalent efficacy.

Transplant 04

A multicentre analysis of the results of kidney transplantation from non-heart-beating donors in the UK

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[1]*Leicester,* [4]*South Thames,* [3]*Newcastle and* [2]*Cambridge Transplant Units,* [5]*UK Transplant Service*

Aims: Whilst non-heart-beating donors (NHBD) have been shown to be a useful additional source of transplant organs, there continue to be concerns about the effects of prolonged warm ischaemic injury. The aim of this multicentre study was to determine the outcome of kidney transplantation from NHBD in the UK.

Methods: Prospectively collected data from four centres (Leicester, South Thames, Newcastle and Cambridge) performing NHBD kidney transplants during the period 1991–2001 was collated. The following outcome measures were assessed incidence of primary nonfunction (PNF transplants that never worked), delayed graft function (DGF: requirement for dialysis in the first post-transplant week) and initial graft function rates (IF), acute rejection episodes, renal function and actual allograft survival rate.

Results: A total of 285 NHBD kidney transplants were performed in the study period. Fifty-one per cent of donors were controlled and 49 per cent uncontrolled. The mean (range) donor age was 44 (6–74) years. The transplanted kidneys suffered a mean (range) first warm ischaemic time of 27 (10–75) min. The rates of PNF, DGF and IF were 15, 68 and 17 per cent, respectively. Acute rejection occurred in 41 per cent of patients in the first year post-transplant. Allograft function and survival rates are shown in the table.

Time of post-transplant (months)	Serum creatinine (mmol L^{-1})	Allograft survival (%)
12	179 (88)	79
36	177 (73)	75
60	197 (101)	69

Conclusions: Renal transplants from NHBD yield acceptable renal function and allograft survival rates over a 5-year period. This audit provides further evidence to support the more widespread introduction of NHBD kidney transplantation.

Transplant 05

The role of reduced-size liver transplantation in an adult transplant programme

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Aim: To review the practice of reduced-size liver transplantation (RSLT) in an adult liver transplant programme.

Methods: Since 1997, we have performed 17 reduced-size liver transplants, resulting in 22 transplants – 17 adults and 5 children. Data were collected prospectively on the unit database and outcome of 'exported' split livers ($n = 5$) established by contact with recipient units.

Results: There were 17 adult recipients (3 men and 14 women) and 5 children. The mean body weight of adult recipients was 51.6 kg and the median waiting time for these adult patients on the routine list was 123 days (range 4–432) compared to 35 days in the patients on the list who received whole livers during this period ($n = 117$). Six patients with fulminant hepatic failure (FHF) were transplanted with seven RSLs. There were three postoperation deaths (3/17 = 18 per cent), all in fulminant patients. The only patient who had primary nonfunction (PNF) was retransplanted with a whole liver and is alive and well (Table 1).

Complication	*n*	%	% in FHF
Bleeding	6	35.3	50
Bile leak	2	11.8	0
Hepatic artery thrombosis	1	5.8	16
Primary nonfunction (PNF)	2	11.8	14.3
Sepsis	13	76.5	10
Hyperacute rejection	1	5.8	16
Mortality	3	18	50

Conclusions: Twenty-two patients benefited from RSLT in an adult programme. Mortality was related to patients' preoperative diagnosis (fulminant hepatic failure), and both mortality and major morbidity in the remaining patients was similar to 'whole organ' liver transplantation. The technique of RSLT should be considered in specific groups of patients who are at a disadvantage on the National Waiting List. If used in FHF, the results are inferior.

Transplant 06

Donor safety in adult-to-adult living donor liver transplantation using right-lobe grafts with middle hepatic vein

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Aim: To review donor safety in a consecutive series of living donor liver transplants (LDLT) using right-lobe grafts, with middle hepatic vein.

Methods: A retrospective review of all LDLT was undertaken from a prospectively compiled database. Right-lobe grafts were retrieved without the application of a Pringle maneuver. Computer tomographic volumetry was used to calculate left- and right-lobe volumes.

Results: Ninety-one living donors (55 female) with a median age of 37 years (18–57 years) donated a right-lobe graft between May 1996 and February 2002. In 90 patients, the graft included the middle hepatic vein. Donor liver median volume was 703.5 mL (450–1160 mL) and weight was 595 g (390–1140 g). The left lobe–total liver volume ratio was 0.339 (0.236–0.452). Total operative time was 524 min (378–932 min). The median blood loss was 466 mL (42–1600 mL). One donor, who was anaemic preoperatively, received a blood transfusion (one unit). Median postoperative hospital stay was 10 days (5–38 days). There was no donor mortality. Overall morbidity was 25 per cent. There were no biliary leaks, although two patients developed bile duct strictures. One required a segment III bypass, the other was managed successfully by endoscopic dilatation. Four patients developed postoperative cholestasis. Two had mild (5 per cent) and two had moderate steatosis (15–20 per cent). All had normal liver function at 6 months.

Conclusion: Live donation of right-lobe grafts with middle hepatic vein for adult-to-adult liver transplantation is safe, provided that the residual liver volume exceeds 30 per cent of the total liver volume and the liver is only mildly steatotic.

ASGBI: Six of the Best, Breast 07–12

Wednesday, 22 May 11.10–12.45

Six of the Best, Breast 07

Surgery does not alter the incidence of circulating tumour cells in the bone marrow of patients with breast carcinoma

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Aims: It has been advocated that surgery may cause shedding of neoplastic cells into the circulation of patients undergoing oncological surgery, thereby facilitating haematogenous spread. Our aim was to investigate if surgery increased the amount of viable tumour cells shed into the circulation by assessing the peripheral blood and the bone marrow of patients using flow cytometry and immunomagnetic separation (IMS).

Methods: Using the technique described above, we analysed the preoperative peripheral blood and bone marrow aspirate, the intraoperative axillary vein blood, and the postoperative bone marrow of 20 patients with breast carcinoma. Using IMS, samples were enriched by coupling FITC-conjugated MNF116 (a pancytokeratin epitope) positive cells to anti-FITC microbeads. They were then subjected to flow cytometry. In all, 124 samples were analysed in this manner.

Results: Of the 20 patients assessed, 17 (85 per cent) had at least one positive sample. The incidence of cytokeratin-positive cells in preoperative samples was 42 per cent (25/59) and in postoperative samples was 46 per cent (24/52). There was a significant correlation between pre- and postoperative sampling (Pearson correlation $P < 0.001$).

Conclusion: Surgery does not alter the incidence of cytokeratin-positive epithelial cells in the peripheral blood or bone marrow of patients undergoing resection of primary breast tumours.

Six of the Best, Breast 08

Preoperative factors do not predict which patients require reoperation for ductal carcinoma *in situ* (DCIS)

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Aims: Over 80 per cent of women have a preoperative diagnosis of DCIS made by core biopsy, but a cohort of patients require reoperation to complete surgical treatment. Preoperative factors which identify these women would reduce the reoperation rate. Our aim was to determine factors predictive for reoperation following breast conserving surgery for core biopsy diagnosed, screen-detected DCIS.

Methods: Consecutive women diagnosed with pure DCIS ($n = 160$) after stereotactic core biopsy were included. A primary therapeutic local excision was attempted in all patients, with completeness of excision assessed by intraoperative specimen X-ray. Risk factors for reoperation were assessed by univariate binary logistic regression.

Results: Occult invasive cancer was found at operation in 64 patients (40 per cent), with 57 women requiring reoperation for either close (<1 mm) or involved excision margins (±axillary staging, 86 per cent) or for axillary staging alone (16 per cent). Pathological and radiological estimates of DCIS size were similar ($r = 0.49$, $P < 0.001$).

Factor	Reoperation (univariate)		
	Odds ratio	95% CI	*P*-value
Grade of surgeon	1.94	0.68–5.60	0.22
Mammographic size	1.01	0.97–1.04	0.60
Core DCIS pathology type	0.84	0.63–1.12	0.23
Core DCIS grade	1.20	0.65–2.24	0.56
Specimen X-ray clear	1.22	0.89–4.76	0.75
Specimen weight	0.99	0.93–1.00	0.70
Operative pathology	0.85	0.59–1.22	0.37
Pathology size	1.04	1.01–1.08	0.01
Excision margin width	0.03	0.0–0.18	<0.001

Conclusions: Preoperative factors and intraoperative assessment of completeness of excision did not predict for reoperation. Despite an increased preoperative core biopsy diagnosis, re-excision after primary "therapeutic" DCIS operations will remain necessary in 40 per cent of patients.

Six of the Best, Breast 09

Assessment and validation of Van Nuys prognostic index in a series of patients with ductal carcinoma *in situ* (DCIS) of the breast

J. Jenkins, C.R. Wilson, E.A. Mallon, W.D. George and J.C. Doughty

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Aims: The Van Nuys prognostic index (VNPI) is a numerical algorithm based on tumour features and recurrence data from a large series of DCIS patients. It attempts to quantify easily measured prognostic factors in a reproducible fashion separating DCIS patients into three groups. The value of the VNPI will be determined by its reproducibility in other institutions.

Methods: A total of 700 DCIS patients were identified from a prospective audit. Clinical and pathological data were assessed and a VNPI score was assigned to each woman retrospectively. Patients with a high score (8 or 9) usually had undergone mastectomy and were not considered in this analysis.

Results: Median follow up was 5.8 years. A total of 423 patients underwent BCT. One hundred and seventy-nine women (42.4 per cent) received DXT. In the BCT group, 80 patients had a score of 3 or 4 with 8 LR (10 per cent). In the BCT/DXT group, 42 patients had a score of 3 or 4 with 1 LR (3 per cent). When a score of 4 was considered alone recurrences were 14 per cent (BCT alone) and 3 per cent (BCT/DXT). In the group with scores of 5, 6 or 7, 115 patients underwent BCT alone with 49 (42 per cent) recurrences. Seventy-six patients underwent BCT/DXT with nine (9 per cent) recurrences.

Conclusion: In this study, the VNPI remains useful in predicting recurrence and guiding therapies. An intermediate score in the BCT alone group in this analysis had a high chance of local recurrence. Adjuvant DXT reduced the recurrence rate. In contrast to other studies, BCT alone patients with a low VNPI (3 or 4) appeared to benefit from adjuvant DXT.

Six of the Best, Breast 10

Impact of preoperative core biopsy on reoperation rates for screen-detected ductal carcinoma *in situ* (DCIS)

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Aims: Stereotactic core biopsy has resulted in an increased preoperative diagnosis of DCIS and has replaced wire-localized open biopsy for screen-detected microcalcification, potentially reducing the need for reoperation to obtain clear excision margins. Our aim was to assess the impact of the introduction of stereotactic core biopsy on the reoperation rate for screen-detected impalpable DCIS.

Methods: A retrospective analysis was performed on a cohort of 221 women with screen-detected pure DCIS with (after 1997, $n = 74$) and without (before 1997, $n = 147$) a preoperative core biopsy diagnosis. An intraoperative specimen X-ray was performed to assess the adequacy of excision. The two groups of patients were matched for age and DCIS nuclear grade.

Results: Pathological and mammographic DCIS tumour sizes were similar ($r = 0.71$, $P < 0.001$).

	Diagnosis		
Variable	Core biopsy	Wire localized	*P*-value*
First procedure (wire localized)	Therapeutic ($n = 74$)	Diagnostic ($n = 147$)	N/A
Patients needing second operation	43%	41%	0.73
Total number of operations (mean)	1.45	1.50	0.75
Clear margins (>1 mm) after first operation	65%	70%	0.43
Median weight of operation specimen	34 g (9–380)	21 g (4–222)	<0.01
Median pathological tumour size	18 mm (4–75)	13 mm (2–40)	<0.001

*Mann–Whitney *U*-test.

Conclusion: Changes from diagnostic to therapeutic primary operative excision for screen-detected DCIS have not resulted in a reduction in the total number of operations required to complete treatment, because mammography underestimates DCIS tumour size in 30 per cent of patients.

Six of the Best, Breast 11

Dynamic contrast-enhanced magnetic resonance imaging significantly improves the surgical management of invasive lobular breast cancer

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Aims: Infiltrating lobular carcinoma (ILC) accounts for 5–10 per cent of all breast cancers and is frequently multifocal and traditional diagnostic methods are unable to reliably detect this preoperatively. The aim of the study was to evaluate the efficacy of current imaging modalities compared with dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) in the evaluation of ILC in the breast.

Methods: Data from women with ILC treated between 1996 and 2000 who had undergone preoperative triple assessment and DCE-MRI were analysed. The diagnostic accuracy and the detection of multifocal disease for each modality was assessed and any changes in clinical management following DCE-MRI were recorded.

Results: Twenty-one women (mean age 57, range 43–72 years) were recruited. Histological diagnosis ($n = 21$) revealed: pure ILC ($n = 5$), ILC + DCIS ($n = 4$), and ILC + LCIS ($n = 12$). The sensitivity of X-ray mammography, ultrasound, clinical assessment and DCE-MRI was 90.5, 87.5, 76.2 and 95.2 per cent, respectively. DCE-MRI identified all the patients with subsequently histologically proven multifocal disease ($n = 11$) with PPV and NPV of 100 and 95.2 per cent, respectively. X-ray mammography and ultrasound combined identified only 27 per cent (3/11) with PPV and NPV of 100 and 55.6 per cent, respectively. Management was changed in 24 per cent (5/21) of the cases following DCE-MRI.

Conclusions: DCE-MRI has a higher sensitivity than other imaging modalities and is able to accurately delineate multifocal disease not evident on conventional imaging. DCE-MRI should therefore be used for accurate staging prior to surgery for invasive lobular breast cancer.

Six of the Best, Breast 12

Do patients with delay in diagnosis of breast cancer have a poor outcome? A case–control study

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Aims: The literature on the effect of delay in diagnosis of breast cancer is contradictory which may be explained by the earlier presentation of more aggressive tumours. The aim of this study was to compare prognostic factors and outcome in patients with and without a delay in diagnosis.

Methods: Patients presenting with an in-hospital delay in diagnosis of breast carcinoma of more than 3 months between August 1971 and October 2000 were matched for age and tumour type with one or two control patients without a delay. The grade of tumour and lymph node status were compared between the two groups and overall survival was compared by Kaplan–Meier life table analysis.

Results: There were 90 patients with a delay in diagnosis (range: 3–66 months). These were matched with 165 patients as controls. The mean age of cases was 51 years *versus* 52 years in controls and the mean follow up was 91 months *versus* 92 months. The control patients with no delay in diagnosis had more severe disease, with more grade 3 tumours (28 per cent *versus* 8 per cent, $P = 0.01$) and a nonsignificant trend to a higher positive node status (30 per cent *versus* 17 per cent, $P = 0.098$ and worse survival ($P = 0.17$, log-rank test). A delay of more *versus* less than 6 months showed no effect on life table analysis.

Conclusion: This case–control study supports the hypothesis that previous paradoxical reports of an improved prognosis in patients with a delay in diagnosis of breast cancer is due to the earlier presentation of patients with more severe disease.

ASGBI: Colorectal 07–16

Wednesday, 22 May 11.10–13.10

Colorectal 07

The outcome of loop ileostomy closure: a prospective study

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Aims: The use of a loop ileostomy is an effective method to protect pelvic anastomoses. Its use has increased recently, although there is some debate as to the routine use of a stoma. A second operation is required to close the stoma, with potential complications. The aim of this study was to assess prospectively the morbidity of closure of loop ileostomy

Methods: All patients scheduled for loop ileostomy closure over a 12-month period were included The operative technique, complications and length of stay were recorded

Results: A total of 50 consecutive patients (28 male, 22 female) with a median age (interquartile range, IQR) of 56 years (42–73) underwent ileostomy closure, at a median time (IQR) of 28 weeks (18–48) after formation Twenty-four (48 per cent) were formed after low anterior resection for carcinoma, 20 (40 per cent) after ileal pouch anal anastomosis for ulcerative colitis and 6 (12 per cent) after other procedures Thirty-eight (76 per cent) had a stapled closure and 12 (24 per cent) sutured Twelve patients (24 per cent) developed complications, 6 (12 per cent) had intestinal obstruction of which one required a laparotomy, 4 (8 per cent) had wound infections of which one required re-operation, 1 (2 per cent) had an ileal anastomotic leak and subsequently died and 1 (2 per cent) died from a myocardial infarction. The median length (IQR) of hospital stay was 8 days (7–10).

Conclusions: We have demonstrated that a quarter of patients develop complications after loop ileostomy closure The majority of these are minor Methods to reduce the number of complications, such as optimum time to closure and distal limb irrigation techniques, need to be studied

Colorectal 08

Topical perioperative application of 0.2 per cent GTN paste protects the anal sphincters from colorectal stapled anastomotic related injury

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Aim: Transanal stapled colorectal anastomosis is associated with internal anal sphincter (IAS) injury and postoperative incontinence A randomized trial was conducted to investigate whether perioperative perineal application of 0.2 per cent GTN paste, which causes IAS relaxation, could prevent such injury

Methods: Fifty patients undergoing cancer resection were randomized between January 1999 and October 2000 to receive perioperative perianal 0.2 per cent GTN paste (23 patients) or placebo gel (27 patients) 20 min prior to their transanal stapled anastomosis Pre- and postoperative (12 months) continence scores, pre- and postoperative transanal ultrasound, ease of insertion of the stapler and perioperative patient blood pressure were recorded.

Results: All patients were continent without ultrasound evidence of anal sphincter defects preoperatively The median height of the anastomosis from the anal verge was 10 cm (range, 7–11) There were no adverse episodes of intraoperative hypotension Six patients in the placebo group had evidence of IAS injury It was more difficult to insert the stapler in the placebo group (difficult: PLACEBO 8 GTN 1; some resistance: PLACEBO 16, GTN 8; no resistance: PLACEBO 3, GTN 14) Postoperative continence scores were better in the GTN group (PLACEBO 5.1 ± 1.1, GTN 3.3 ± 0.7, $P = 0.01$, Fishers Exact Test).

Conclusion: Perioperative perineal 0.2 per cent GTN paste application protects anal sphincter integrity and postoperative function

Colorectal 09

The resting pressure gradient (RPG) is an early indicator of faecal incontinence

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Aims: Incontinence scores are used to quantify faecal incontinence and allow comparison between patients Anorectal manometry is routinely used for the assessment of incontinence Patients with mild incontinence often have normal results We have reported the measurement of RPG in the assessment of incontinence. This study examined the role of this technique in patients with varying degrees of incontinence

Methods: Patients referred for anorectal investigations were recruited Patients completed a Wexner incontinence score (WIS) Patients were grouped by incontinence score continent (WIS 0, $n = 37$), mildly incontinent (WIS 1–7, $n = 29$), moderately incontinent (WIS 8–13, $n = 34$) and severely incontinent (WIS 14–20, $n = 16$) Patients underwent standard anorectal manometry The following manometric measurements were recorded maximum mean resting pressure (MMRP), maximum mean squeeze pressure (MMSP), vector volume (VV) and resting pressure gradient (RPG) The Mann–Whitney *U*-test was used to analyse results.

Results: A total of 116 patients were recruited into the study [92 females, 24 males: median age 51 (interquartile range 37–67) years] Each measurement was compared between the continent group and. (a) all incontinent patients (b) moderately and severely incontinent patients and (c) mildly incontinent patients

	RPG	MMRP	MMSP	VV
Continent *vs* group (a)	0.005*	0.001*	0.021*	0.004*
Continent *vs* group (b)	0.008*	<0.001*	0.001*	<0.001*
Continent *vs* group (c)	0.029*	0.217	0.877	0.356

Values are *P*-values *indicates significance

Discussion: This study shows that RPG is an early indicator of faecal incontinence Standard measurements of MMRP, VV and MMSP are often normal in patients with mild incontinence and only become significantly lower in patients with at least moderate symptoms We recommend the routine use of RPG in the manometric assessment of faecal incontinence

Colorectal 10

The role of transanal endoscopic microsurgery in the management of malignant rectal tumours

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Aims: The role of local resection for benign rectal disease is well established, however, controversy still exists regarding its use in the treatment of malignant tumours The aim of this study is to evaluate the role of Transanal Endoscopic Microsurgery (TEM) in the management of rectal tumours, in particular cancers

Methods: A total of 56 successive patients, mean age of 68 (range 37–91) years, underwent TEM by a single surgeon for rectal tumours over a 5-year period. Data were collected prospectively and reviewed with regards to diagnosis, TNM stage, length of operation and hospital stay, resection margins and recurrence rates

Results: Mean follow up was 21 months (range 3–60) 37 patients had benign rectal tumours and 19 had cancers of which 7 were T1, 9 T2, and 3 were T3 Mean length of surgery was 83 min (range 43–180) and mean hospital stay was

3.6 days (range 1–10). Margins were clear in 54 patients (96 per cent). Involved margins were observed in one patient with benign disease and one patient with rectal carcinoma, who had a palliative resection and died of metastatic disease. Hospital mortality was zero and only minor morbidity was observed in 23 per cent, which included bleeding, transient faecal incontinence and urinary retention. Recurrence rates for T1 and T2 tumours were 0 and 43 per cent, respectively.

Conclusions: TEM for both benign and malignant disease is safe with zero mortality and low morbidity. However, its use in the surgical management of rectal cancer should be limited to those with early disease (T1) or for palliation.

Colorectal 11

Magnetic resonance imaging remains an imprecise tool for staging rectal cancer

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Aim: Long and short course preoperative radiotherapy are increasingly employed for the treatment of patients with rectal cancer and magnetic resonance imaging (MRI) has been advocated as the gold standard for staging these patients as part of their treatment planning process. We have prospectively evaluated the value of MRI in staging rectal cancer patients.

Methods: A total of 45 consecutive patients undergoing surgery for rectal cancer were assessed. Each underwent preoperative spiral CT of their lungs, liver and pelvis combined with pelvic MRI (1.0T Whole Body Siemens Harmony System with a phased array coil). Tumours were staged preoperatively by MRI/CT using the Dukes' and TNM systems and these results were compared with formal histopathology. Circumferential resection margins were additionally predicted radiologically and compared with actual histology.

Results: Histopathology assessment revealed there to be 10 patients with a Dukes' A tumour, 19 Dukes' B and 16 with stage Dukes' C. MRI understaged four patients, overstaged 14 and correctly staged 27 (sensitivity 60 per cent). Radiological assessment of circumferential resection margins was hampered for patients with anterior tumours and lateral tumours with an anterior component because of blurring of the mesorectal margin.

Conclusions: MRI remains an as yet insensitive method of staging rectal cancer. The images obtained in many patients prevents useful analysis of predicting circumferential resection margins.

Colorectal 12

Prognostic significance of elevated plasma big endothelin-1 levels in patients with colorectal cancer

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Aims: Clinical, laboratory and pathological variables in patients with colorectal cancer may identify high-risk groups to whom beneficial adjuvant therapy could be given. This prospective study was performed to determine whether preoperative plasma big ET-1 levels might be a useful as a prognostic indicator in patients with colorectal carcinoma.

Methods: Sixty-five consecutive patients with colorectal cancer confirmed by biopsy were entered prospectively into this study over a 12-month period. Plasma samples from a peripheral vein were obtained prior to surgery and were analysed by using a single step sandwich enzyme immunoassay (Biomedica, Austria). Univariate analysis of survival using age ($<$ or >70 years), sex, Dukes' stage (A & B *versus* C), tumour size ($<$ or >50 mm), vascular invasion and plasma big ET-1 levels were performed and significant factors were then analysed in a multivariate Cox regression model.

Results: Three variables, age, Dukes' tumour stage and plasma big ET-1 levels were found to have a prognostic significance ($P < 0.05$). Factors associated with a poorer prognosis were age more than 70 years ($P = 0.02$), Dukes' C tumours ($P = 0.04$) and plasma big ET-1 levels more than 4.2 pg/mL ($P = 0.02$). The Cox regression model identified the same three variables as having independent prognostic value for survival.

Conclusion: Preoperative plasma big ET-1 levels may be useful in predicting overall survival in patients with colorectal cancer. In particular, plasma big ET-1 levels may be useful in the selection of high-risk lymph node negative patients with colorectal cancer for adjuvant therapy.

Colorectal 13

The impact of colorectal subspecialization on outcomes for rectal cancer: a 5-year review

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Aim: Subspecialization is intended to improve surgical and oncology related outcomes in cancer patients. This study analysed the impact of colorectal subspecialization on rectal cancer outcomes in a unit with four existing experienced general surgeons.

Methods: A 5-year review of surgical and oncological outcomes in 207 consecutive patients was conducted. The results were reviewed externally.

Results: A total of 127 procedures were performed by two colorectal surgeons (CRS) and 80 by four general surgeons (GS). Permanent stoma rates (CRS 21 per cent, GS 29 per cent; $P = 0.5$, 95 per cent CI 0.4–1.47), postoperative morbidity (CRS 43 per cent, GS 35 per cent; $P = 0.47$; 95 per cent CI 0.7–2.2) and 30 day mortality (CRS 5 per cent, GS 6 per cent; $P = 0.75$, 95 per cent CI 0.22–2.5) were comparable between CRS and GS. Local recurrence rates (CRS 13 per cent, GS 15 per cent; $P = 0.68$, 95 per cent CI 0.37–1.68) and the risk of developing disseminated disease (Odds Ratio 0.72; $P = 0.38$, CI 0.37–1.22) were also similar between the two surgeon groups.

Conclusions: Colorectal subspecialization has not resulted in improved crude measures of surgical and oncological outcomes for patients with rectal cancer. The decision of individual units to subspecialize should consider local results through careful audit in relation to national guidelines.

Colorectal 14

Resection or radiofrequency ablation for solitary colorectal liver metastases?

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Aim: Liver resection is the accepted treatment of choice for patients with solitary colorectal liver metastases. In recent years, however, the role of radiofrequency ablation has occupied an increasingly prevalent role. In the absence of randomized controlled trials this study aims to compare outcome in patients with solitary colorectal liver metastases treated by surgery and radiofrequency ablation.

Methods: Twenty-five patients with solitary colorectal liver metastases were treated by radiofrequency destruction (mean age 57, 34–80). The indications were extrahepatic disease in 7, vessel contiguity in 9 and comorbidity in 9. Outcome measures were compared with 20 patients treated by appropriate liver resection for solitary metastases. Most patients in both groups received systemic chemotherapy in addition.

Results: The median survival following liver resection was 40.6 months (range 0–97 months) with a 3-year survival of 55 per cent. There was one postoperative death and morbidity was minimal. Median survival following radiofrequency was 37 months (range 9–67 months) and 3-year survival of 52 per cent.

Conclusions: Resection and radiofrequency ablation of solitary metastases result in apparently comparable survival. The latter is less invasive and requires either an overnight stay or day case facilities only. A prospective randomized controlled trial comparing these two modalities is indicated.

Colorectal 15

One hundred consecutive cases, predominantly of pseudomyxoma peritonei, referred to a peritoneal surface malignancy unit: operability and early outcomes

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Aims: Peritoneal malignancy is common and almost inevitably fatal in many intra-abdominal cancers. A small proportion of patients with peritoneal surface disease, particularly with pseudomyxoma peritonei (incidence one per million per year and almost always from an appendix primary) may be amenable to complete cytoreductive peritonectomy with good palliation, long-term disease free survival and possibility of cure.

Methods: One hundred consecutive referrals (1994–2000) to a peritoneal surface malignancy unit were received between January 1994 and August 2001. Assessment of suitability for complete tumour removal and intraperitoneal chemotherapy was made by CT and clinical examination. Sixty-two of 100 underwent laparotomy (54 pseudomyxoma peritonei, 4 adenocarcinoma, 3 mesothelioma and 1 leiomyoma).

Results: Complete tumour removal was achieved in 38/54 pseudomyxoma peritonei (17 males), 2/3 mesothelioma, 2/4 adenocarcinoma and 1/1 leiomyoma Major palliative debulking was achieved in 13 pseudomyxoma and 1 mesothelioma Three patients with pseudomyxoma and 2 with adenocarcinoma were inoperable. Median operating time for the 43 completed cases was 10.5 (3–18) hours, with median ITU and hospital stay of 7 and 24 days, respectively There were 3/43 (7 per cent) postoperative deaths (2 multi-organ failure and 1 pulmonary embolus)

Conclusions: Just over half the patients referred (62 per cent) underwent surgery and 69 per cent of these had complete cytoreduction. Surgery is radical, time consuming and expensive, and requires careful patient selection to optimise outcomes

Colorectal 16

Laparoscopic surgery for recurrent Crohn's disease

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Aims: The role of laparoscopic surgery for Crohn's disease remains to be defined, although increasingly difficult cases are being treated using minimally invasive techniques. The aim of this study was to clarify the feasibility of laparoscopic surgery for recurrent Crohn's disease

Methods: Between 1994 and 2001, 57 laparoscopic operations were attempted in 48 patients (39 males and 9 females; mean age: 30 years) with ileal or ileocolonic Crohn's disease. Of these, 15 operations were performed due to recurrence at the anastomotic site (recurrent group). The remaining 42 operations were performed as a primary case (control group) The median follow-up was 45 months (range. 1–87 months)

Results: The median time to reoperation was 46 months The incidence of patients with enteric fistulas did not differ between the two groups (recurrent group 6/15, 40 per cent vs control group 23/42, 55 per cent). Conversion to open procedure occurred in two attempts in the recurrent group (2/15, 13 per cent) and three in the control group (3/42, 7 per cent) without any significant differences Operative blood loss and time did not differ between the two groups (recurrent group: 80 mL and 210 min, control group 60 mL and 180 min) There were no differences between the two groups in terms of the postoperative complications (recurrent group: 3/15, 20 per cent vs control group 6/42, 14 per cent) and hospital stay (recurrent group 8 vs. control group: 8 days) There were no deaths in either group.

Conclusions: Laparoscopic surgery for recurrent Crohn's disease is feasible in selected patients without increasing operative time, conversion rate and postoperative complications.

SARS: Patey Prize 01–08

Wednesday, 22 May 10.10–12.45

Patey Prize 01

Reflux-related oesophageal mucosal injury: relationship to transforming growth factor beta (TGF-β) expression

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Background: Overexpression of TGF-β has been linked to the occurrence of excessive fibrosis in both surgical skin wounds and in renal allografts after transplantation. Its involvement in the pathogenesis of gastro-oesophageal reflux disease has not been reported.

Methods: Endoscopic oesophageal biopsies were obtained from control subjects free of reflux symptoms ($n = 4$), patients with reflux symptoms but no oesophagitis ($n = 4$), patients with grade 2 oesophagitis ($n = 4$), patients with oesophageal strictures ($n = 4$) and patients with Barrett's oesophagus ($n = 4$). A lightcycler was employed to perform real time quantitative RT-PCR for TGF-β on the biopsies.

Results: The table indicates the level of TGF-β expression according to the endoscopic findings. The values indicated are median and range.

Grade of oesophagitis	Control	Grade 0	Grade 2	Stricture	Barrett's
*TGF-β levels × 10^3	5.3 (1.1–28)	15 (10–56)	18 (1.5–42)	41 (18–71)†	1.9 (0.93–3.4)‡

*copies/25 ng RT RNA.
†$P = 0.06$ *versus* controls and $P = 0.03$ *versus* Barrett's, ‡$P = 0.03$ *versus* grade 0 (Mann–Whitney *U*-test).

There was an increase in the level of TGF-β expression as the severity of mucosal injury progressed, so that patients with oesophageal strictures had an eight-fold higher level of TGF-β expression than control subjects. Patients with Barrett's oesophagus had the lowest levels of TGF-β expression of all patient groups.

Conclusions: These findings indicate that excess TGF-β expression is related to the genesis of fibrosis in the oesophagus as a consequence of gastro-oesophageal reflux.

Patey Prize 02

Sacral nerve neuromodulation for the treatment of resistant faecal incontinence

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Background: Faecal incontinence is common and associated with a high level of physical and social disability. If conventional treatment fails the remaining options are limited. Sacral nerve stimulation, involving chronic neuromodulation of the sacral nerves, is a novel treatment for this condition. We report our 5-year experience.

Methods: Seventeen patients (16 women, median age 59 [37–71]) were implanted with initial temporary, and then permanent stimulators. All had severe incontinence, resistant to maximal conventional treatment, including behavioural therapy. The median preoperative duration of symptoms was 6 years (2–15). Aetiology included obstetric injury ($n = 7$), scleroderma (4), idiopathic (3), repaired rectal prolapse (2) and fistula surgery (1). Clinical evaluation, endoanal ultrasound, bowel diary, quality of life (SF36) and anorectal physiology were completed before and after treatment.

Results: At median follow-up of 26 months (3–62) continence improved significantly in all patients. The median weekly incontinent episodes decreased from 12 (2–30) to 0 (0–7), $P < 0.001$. Twelve of 17 were fully continent. Urgency improved in all; (mean [standard deviation] ability to defer <1 min [0.5] *versus* 8 [5], $P < 0.01$). Maximal anal resting pressure increased during temporary stimulation (35 cm H_2O [17] *versus* 49 [21], $P < 0.05$) and maximal squeeze pressure with permanent stimulation (44 [40] *versus* 69 [49], $P < 0.01$). Rectal threshold sensation to distension improved (47 mL air [19] *versus* 35 [15], $P < 0.05$). Other physiological variables did not change significantly. Quality of life improved in all, $P < 0.05$. There were no major complications.

Conclusions: Sacral nerve stimulation is a safe and effective treatment for faecal incontinence when other treatments have failed.

Patey Prize 03

MMP-8: A novel collagenolytic pathway in acute plaque disruption

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Background: Loss of interstitial collagen, especially type I collagen, renders atherosclerotic plaques prone to rupture. Subsequent thrombosis and embolism may cause stroke and myocardial infarction. Initiation of collagen breakdown requires interstitial collagenases, a matrix metalloproteinase (MMP) subfamily including MMP-8. The aim of this study was to quantify the level of MMP-8, the enzyme that preferentially degrades type I collagen, in carotid plaques, and to correlate this with features of plaque instability.

Methods: Plaques were retrieved from 75 consecutive patients undergoing carotid endarterectomy. MMP-8 levels were quantified using ELISA. Histological plaque sections were assessed blindly for the presence of plaque rupture and immunostaining was performed for MMP-8. All patients underwent preoperative transcranial Doppler monitoring to detect spontaneous embolization. Results expressed as median MMP-8 concentration ng mL^{-1} (IQR).

Results: The plaque level of MMP-8 was significantly higher in patients with recent symptoms (symptoms <4 weeks), histological plaque rupture and spontaneous embolization (see Table). There was a significant correlation between MMP-8 and MMP-9 levels, a gelatinase known to be associated with plaque instability. Immunohistochemistry revealed staining for MMP-8 in areas of intense inflammatory infiltration.

	Positive	Negative	*P*-value
Recent symptoms	0.54 (0.15–1.59)	0.10 (0–0.49)	0.03
Plaque rupture	1.25 (0.19–3.05)	0.08 (0–0.41)	0.009
Embolization	0.44 (0.15–1.33)	0.07 (0–0.41)	0.004

Discussion: The MMP-8 level is significantly higher in unstable plaques based on symptoms, histology and spontaneous embolization. Together with MMP-9, these enzymes have the potential to degrade all components of the extracellular matrix upon which plaque integrity depends, and represent a target for pharmacotherapy to prevent stroke.

Patey Prize 04

Pirfenidone, a novel antifibrotic agent, attenuates intimal hyperplasia via its potent antiproliferative effect on vascular smooth muscle cells

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Aims: Intimal hyperplasia is characterized by vascular smooth muscle cell proliferation, migration and deposition of extracellular matrix. Pro-fibrotic cytokines, matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) are pivotal mediators in this process. Secretion of metalloproteinases (MMPs) degrade ECM and facilitate migration, whilst tissue inhibitors of metalloproteinases (TIMPs) favour accumulation of ECM. The antifibrotic agent pirfenidone inhibits transforming growth factor-β and MMP synthesis in nonvascular inflammatory models: its effect on injured vascular cells has not been previously reported.

Methods: Sprague–Dawley rats received standard diet or diet supplemented with pirfenidone powder (250, 500, 1000 $\mathrm{mg\,kg^{-1}}$ per day). Animals underwent left common carotid artery balloon angioplasty and were explanted at 14 days. Morphometric analysis was performed on transverse sections and intima media ratios calculated. Extracellular matrix accumulation was quantified using picrosirius red staining. Pro-fibrotic gene expression was measured with RT-PCR.

Results: Neointimal thickness was significantly reduced in a dose-dependent manner; Control, median values 1.63 (range 1.44–1.99), Pirfenidone 250 $\mathrm{mg\,kg^{-1}}$ 0.73 (0.65–1.26; $P < 0.005$), Pirfenidone 500 $\mathrm{mg\,kg^{-1}}$ 0.48 (0.12–0.85, $P < 0.001$), Pirfenidone 1 $\mathrm{g\,kg^{-1}}$ 0.009 (0–0.28; $P < 0.001$). Expression of MMP-2, MMP-9, TIMP-1, collagen III and TGF-β were all significantly inhibited at 14 days. Collagen III expression was significantly reduced at 14 days ($P < 0.001$) and was consistent with a reduction in intimal extracellular matrix deposition at 14 days ($P < 0.002$).

Conclusions: Pirfenidone attenuates intimal hyperplasia and significantly inhibits expression of metalloproteinases governing smooth muscle cell proliferation and migration (MMP-2 and 9), and genes favouring ECM accumulation (TIMP-1 and collagen III). Pirfenidone may have a role in the prevention of intimal hyperplasia and attenuate the development of chronic rejection in solid organ allografts.

Patey Prize 05

Selective estrogen receptor modulators (SERMS) inhibit the effects of insulin-like growth factors in hyperparathyroidism (HPT)

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Background: Primary HPT predominately affects females and has a peak incidence at the perimenopausal period. This has led to speculations that an estrogen imbalance may have a role in this condition. We have previously demonstrated the importance of the insulin-like growth factor (IGF) axis in HPT. The SERM, tamoxifen (tam) has been shown to modulate the IGF axis. We propose to examine the interactions of SERMs and IGF in a primary parathyroid cell culture model.

Methods: Estrogen (E2) receptors were evaluated by Western immuno-ligand blotting. Sixteen parathyroid glands from 14 patients were included. Following adhesion, the cells were transferred to serum free media and dosed once with IGF (I or II) ± estrogen ± SERMs (Tam), faslodex (fas, ICI 182780)) for 96 h. Proliferation was assessed by measuring tritiated thymidine incorporation.

Results: Both primary (1) and secondary (2) HPT express α and β ERs. 1HPT and 2HPT had comparable responses and were analysed together. Compared with control (100%), IGFs (I & II) induced a significant increase in DNA synthesis. E2 at 10^{-8} and 10^{-7} M (physiological range) inhibited DNA synthesis but had no significant effects on IGF (I & II) ($P > 0.05$). Tam inhibited basal DNA synthesis ($P < 0.05$) and abolished the effects of both IGF I and II ($P < 0.05$). To clarify whether these effects can be ascribed to Tam's ER antagonist property, Fas (a pure ER antagonist) was examined. Fas at 10^{-7} M inhibited both IGF I and II ($P < 0.05$).

Conclusions: Tam and Fas are capable of reducing basal and IGF stimulated DNA synthesis. There are obvious implications for cancer biology as well as therapeutic potential for SERMs in HPT.

Patey Prize 06

Serum matrix metalloproteinases are increased in ruptured aortic aneurysms

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Background: Rupture of an abdominal aortic aneurysm (AAA) carries a mortality rate of up to 90%. Matrix metalloproteinases (MMPs) play a fundamental role in aneurysm formation. Previous studies have suggested a systemic increase in proteolysis in patients with AAAs and MMPs have been shown to be elevated in the serum of these patients. However, no study as yet has demonstrated differences in asymptomatic and ruptured aneurysms. The aim of the study was to determine the MMP profiles of serum from patients presenting for elective and emergency aneurysm repair.

Methods: Samples of venous blood were obtained intraoperatively from 61 patients undergoing elective repair of nonruptured AAA ($n = 50$, median age 71 years), or emergency repair ($n = 11$, age 72) of ruptured AAA. Serum was quantified for the concentration of MMPs-1, -2, -3, -9, -13, TIMP-1 and -2 utilizing ELISA.

Results: There were no significant differences in MMPs-2,-3,-13 and TIMP levels in the serum of patients with nonruptured and ruptured AAA. Serum MMP-1 levels were significantly increased in rupture (10.36 $\mathrm{ng\,mL^{-1}}$ [IQR 5.94–15.94] *versus* 24.81 $\mathrm{ng\,mL^{-1}}$ [18.14–41.39], $P < 0.0001$). MMP-9 levels were six times higher in serum from ruptured compared with nonruptured AAA patients (19.13 $\mathrm{ng\,mL^{-1}}$ [9.27–34.24] *versus* 117.9 $\mathrm{ng\,mL^{-1}}$ [30.43–161.8], $P = 0.0015$).

Conclusions: These data demonstrate that the serum MMP profile of patients with ruptured aneurysms is significantly different to patients with nonruptured AAA. Extension of these data may allow prediction of rupture rates from serum MMP levels.

Patey Prize 07

A novel functional estrogen receptor assay (FERA)

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Background: Expression of estrogen receptor (ER) is used to indicate response to endocrine therapy. However, assays, e.g. immunohistochemistry (IHC), that measure ER and ER regulated progesterone receptor (PR), predict response in only 40–60% of cases. We have developed a functional assay (FERA) based on interaction of ER with estrogen response elements (EREs). The assay uses adenovirus containing 2 EREs linked to a lac-Z reporter (ERE-lacZ).

Methods: Breast cancer cells were purified from 29 surgical specimens obtained from 24 patients. The cells were infected with ERE-lacZ and cultured in medium containing 10 nM estrogen (E2), 100 nM tamoxifen (OHT), or 100 nM ICI 182780 (ICI, a complete ER antagonist). A colorimetric assay was used to assess reporter activity. The results of FERA were compared with IHC.

Results: Eleven patients were ER+/PR+, five were ER+/PR– and eight were ER–/PR– by IHC. Three ER+/PR+ and one ER–/PR– were recurrent cancers and all except one patient (ER+/PR+) had received OHT previously. Using FERA, in 12/16 ER+ and 1/8 ER– cancers, E2 induced a 2–10-fold increase in reporter activity. OHT and ICI were inhibitory in primary cancers. Three patients with ER+ cancer showed no ER activity. Of the recurrences, 2 ER+ cancer showed agonistic activity with OHT and in one this was greater than with E2. ICI remained inhibitory.

Conclusions: ER activity can be reliably assessed using FERA. Three ER+ patients who may not benefit from OHT and one ER– patient who may benefit were identified. In addition, resistance to OHT was demonstrated. FERA indicates that ICI (as Faslodex) may be beneficial in these cases.

Patey Prize 08

Further evidence for increased immunogenicity in colorectal cancers expressing microsatellite instability

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Background: Microsatellite instability (MSI) occurs in 15–20% of colorectal cancers and confers a survival advantage independent from tumour stage. This might reflect an improved immunological response against such tumours

Previously we have used real-time RT-PCR to demonstrate that MSI+ tumours show increased expression of activated T-cell markers. By extending this work to protein expression we can now gain a clearer understanding of the immune environment within these tumours.

Aims: To test the hypothesis that MSI+ colorectal cancers elicit an increased antigen-specific immune response compared with MSI-cancers.

Methods: We identified 25 MSI+ and 75 MSI– colorectal cancers, matched for patient age and tumour location. Immunohistochemistry was performed on paraffin embedded sections for T-cell markers, CD3, CD4 and CD8, the specific activation marker, CD25 and the effector protease Granzyme B (GZMB). Intraepithelial lymphocyte (IEL) counts per unit area were scored by a single observer blinded to microsatellite status and independently verified by a consultant pathologist. MSI+ and MSI– groups were compared with a non-parametric test (Mann–Whitney, two-tailed) and significance taken at the 5% level. Results presented as (median, 5th–95th percentiles).

Results: MSI+ tumours had both higher CD3+ and CD8+ than MSI– tumours. Additionally, MSI+ tumours expressed higher CD25+ IEL's and GZMB levels (see Table).

	MSI+	MSI–	
CD3	14.6 (1.0–58.5)	3.9 (0.1–21.8)	$P = 0.003$
CD8	18.8 (0.7–139)	3.3 (0.6–19.0)	$P = 0.008$
CD25	1.5 (0.3–9.5)	1.1 (0.1–2.7)	$P = 0.017$
GZMB	23.5 (5.2–78.0)	6.3 (1.0–24.2)	$P = 0.02$

Conclusions: These results support the hypothesis that patients with MSI+ colorectal cancers exhibit increased antigen-specific immunological activity. Clearly, these findings may underscore the improved patient survival in MSI+ colorectal cancer.

General Papers 07

Surgical dexterity after a night out on the town

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Aims: We studied the effect of binge drinking on surgical dexterity immediately after and the morning after alcohol intake

Methods: Five surgeons (all males, age 31–42 years) were trained on laparoscopic surgical simulator (MIST-VR) to reach a desired standard of performance Three sets of experiments were a control with no alcohol and full night's sleep, a sham night out (sleep deprived) without alcohol and a night out with alcohol ad libitum. Parameters recorded were average time taken to perform task, error rate, average diathermy time and diathermy (damage) time to main object Comparisons between baseline readings, those in the middle of night and those of following morning were made with Wilcoxon signed ranks test (all two tailed, significance at $P < 0.01$).

Results: The candidates consumed on an average 10.33 (range 6–15) units of alcohol and had 0.86 per cent (range 0.71–1.1 per cent) of breath alcohol levels and had an average of 3.75 (range 3–5 h) hours of sleep after a typical night out. The morning-after breath alcohol levels were 0 per cent. There was significant deterioration in the performance indicators in all the parameters immediately after alcohol consumption. The time taken to complete task and the time when the diathermy was used were still significantly worse the morning after the typical night out. The sham night out showed deterioration only on time parameters with control night out showed no changes

Conclusions: A typical night out deteriorates performance on a surgical simulator, which persists till the following morning as compared to just a 'nonalcoholic' night out.

General Papers 08

Problems in providing an emergency laparoscopic cholecystectomy service for acute cholecystitis

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Aims: The high morbidity (28.5 per cent readmission rate) experienced whilst waiting for laparoscopic cholecystectomy (LC) after an index admission with acute cholecystitis (AC) has been reported. Since this report a provision for emergency LC service has been made available in the same DGH This study performed over a 12-month period (October 2000–September 2001) reports the impact of those changes on the management of AC and allows comparisons between these results and those achieved in the previous study (the control period)

Methods: During the study 146 patients presented as emergency with AC Emergency cholecytectomy was performed in 75 and the rest were waitlisted. GI surgeons performed 76 per cent of the LC whilst the non-GI surgeons listed the majority of their cases for delayed LC Seventy-one per cent of the LC was done laparoscopically with an 18.8 per cent conversion rate, 59 per cent (44/75) cholecystectomies were performed by trainees under supervision. The number of emergency LC, conversion rates, complications, median length of total (TLOS) and postop hospital stay (PLOS) were used as end points

Results: There was a seven-fold (Table) increase in the number of cholecystectomies during their index admission in the study group Major complications and conversion rates of LC performed in the study period (5.3 and 18 per cent) were similar to those of delayed LC in the control period (6.2 and 12 per cent) Although the TLOS was less in the study group compared to the control (table), patients with AC still had a median wait of 5 days between their admission and their cholecystectomy

	No	Open chole	LC	Conversion	Complications (major)	TLOS (days)	PLOS (days)
Study-1 year-LC (emergency)	75	22	43 (57%)	10 (18.4%)	5.3%	8	3
Control –2 years (delayed emergency)	141 (119 + 22)	31	98 (69%)	12 (11%)	6.3%	10	3

Conclusions: These results document that emergency cholecystectomy service can be provided with minimal complications and a shorter hospital stay Further improvements to reduce inhospital stay require prompt ultrasound examination and rostering of laparoscopically competent surgeons.

General Papers 09

Laparoscopic appendectomy: a basic surgical trainee procedure?

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Aims: Laparoscopic appendicectomy is a safe and effective procedure but concerns have been raised that the operation has taken appendicectomy away from the basic surgical trainee (BST) and into the hands of more senior surgeons (HST) We assessed the impact of laparoscopic appendicectomy as a trainee operation

Methods: This was a prospective audit of 185 consecutive patients with a clinical suspicion of appendicitis who were referred to a major teaching hospital over a 7-month period Outcome measures included operative time, conversion from laparoscopic to open, complications, and length of stay.

Results: Of the 120 patients who underwent surgical intervention by trainees, six had formal laparotomies, 40 patients successfully had a laparoscopic appendectomy, nine were converted (18.3 per cent), 24 patients had a diagnostic laparoscopy and 41 underwent open appendectomy The performance of BSTs and HSTs is shown below.

	Operation			
Trainee	Diag Lap	Lap App	Converted	Open App
HST	13	21	7	27
BST	11	19	2	14

No significant difference was found in operative time for laparoscopic appendicectomy between trainees (BST median 65 min, HST median 60 min) The duration of laparoscopic appendectomy was significantly longer (median 60 min *versus* 40 min for open ($P < 0.0001$)). Length of stay was significantly shorter in the laparoscopic group compared to open (49 h *versus* 72 h ($P < 0.01$)). There was no difference between the two groups of trainees. The complication rate was lower in the laparoscopic appendectomy group

Conclusion: In our experience laparoscopic appendectomy may fulfil a role in the training of laparoscopy that open appendectomy has traditionally provided for laparotomy

General Papers 10

The learning curve of a simulated surgical task using the Da Vinci telemanipulator system

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Background: Robotic-enhanced surgery has emerged as a solution to some of the drawbacks of traditional minimal access surgery. Master–slave telemanipulator systems have been extensively used in surgical interventions, but the learning curve in this technology has not been objectively assessed

Aims: Delineate the learning curve of surgeons for a robotic task in a complex bench model Find if there is a quantifiable learning process in a short period of exposure to the Da Vinci system.

Methods: Nine surgeons completed five synthetic small bowel anastomoses (Limbs and things, UK) using the Da Vinci robotic system (Intuitive Surgical, CA) in standardized conditions All procedures were digitized and quality scored independently by three surgeons employing the objective structured assessment of technical skills (OSATS) Wilcoxon signed ranks tests were used to compare the first and last attempts. A P-value < 0.05 was considered significant. Cronbach's-alpha was used to measure interrater reliability.

Results: A learning curve was constructed using time and OSATS score of every task. Both variables showed a rapid and progressive learning process. Mean times for the first and last attempt were 3507 s and 2287 s ($P < 0.008$) and mean OSATS scores were 17 and 24 ($P < 0.012$), respectively. A high inter-observer reliability was found ($\alpha = 0.894$).

Conclusions: This study clearly shows a significant reduction in the time taken (34 per cent) and improvement in quality (29 per cent) between the first and fifth attempt in a bench model. Therefore, we conclude that Da Vinci system allows fast adaptation and short learning process regardless previous surgical experience.

General Papers 11

A prospective blind randomized trial comparing collagen mesh with polypropylene mesh in primary inguinal hernia repair: an interim report

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Aims: Tissue repair of primary inguinal hernia using mesh relies on scar tissue bonding the mesh to give a sound repair. Collagen has the theoretical advantage of using natural collagen which will be incorporated into the repair and not be a permanent foreign body.

Methods: A total of 140 patients were prospectively randomized to receive either polypropylene or collagen mesh. Performance was compared at operation, postoperatively, 3 months, 12 months. Patient's perceptions and quality of life were measured. Patients and researchers were blinded and analysis was undertaken at a separate institution.

Results: A total of 78 patients received collagen and 62 received polypropylene mesh, with no difference in handling, insertion and suturing at operation. Postoperatively there was an increase in haematoma in the polypropylene group ($P = 0.048$). Infection and inflammation were similar postoperatively and at 3 months. There was one recurrent hernia in each group at 1 year. Activity and pain scores postoperatively were similar and were normal by 4 weeks. Quality of life was reduced in both groups at 4 weeks: Euroqol $P = 0.002$, SF 36: physical function $P = 0.016$, social function $P = 0.003$. At 3 months there was reduced physical function in the polypropylene group ($P = 0.037$).

Conclusions: Initial results showed that collagen mesh is an effective method of providing tissue repair in primary inguinal hernia. The collagen mesh produced fewer haematomas postoperatively and patients recovered physical activity more quickly. Otherwise it produced results comparable to polypropylene without the risks of a permanently implanted foreign body. It is proposed to recruit 200 patients to the study and undertake follow-up for 2 years postoperatively.

General Papers 12

Current management of localized abdominal sepsis – radiological drainage recommended

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Aims: A radical shift in management of abdominal sepsis from surgery to radiological drainage occurred in 20 years. This is safe and in majority it avoids the need for conventional surgery. When surgery is indicated it provides the roadmap by showing the exact nature of the abscess and its contents. This is a retrospective study from single centre to look at the outcome of management of localized intra-abdominal sepsis by using radiological guided drainage.

Methods: All the patients who underwent radiological intervention for abdominal sepsis between 1995 and 2001 were included in the study. These were scanned and drains were inserted under radiological guidance under local anaesthetic. All these patients were followed up with antibiotics and drainograms when appropriate.

Results: These patients were between ages of 14 and 84 years. A total of 216 procedures were performed. Twenty-four of them were diagnostic in that the patients were subjected to laparotomy or conservative management. A total of 192 patients had therapeutic aspiration or drainage. One hundred and forty-five patients had catheters inserted for continuous drainage and the rest had therapeutic aspiration. These patients included pelvic abscess (15 per cent), perinephric collections (12.4 per cent), subdiaphragmatic abscess (12 per cent), liver abscesses (8 per cent), pseudocysts (7 per cent), pericolic abscess (7.5 per cent), sub-hepatic abscess (5 per cent), psoas abscess (3.4 per cent), appendicular abscess (3.4 per cent), empyema gall bladder and pancreatic abscesses. Complications were minimal including transient sepsis, residual collection, haemorrhage into the cavity and persistent sinus. Our results include bacteriological studies of aspirates, which will be presented in detail at the time of presentation.

Conclusion: Percutaneuos guided aspiration and drainage is a safe and effective alternative to conventional open surgical drainage. We recommend this as the first line of management.

General Papers 13

The management of liver trauma in a tertiary referral centre

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Aims: Historically, surgical intervention for liver trauma has been the accepted method of treatment with an associated high percentage of nontherapeutic laparotomies however, conservative management is now advocated in the haemodynamically stable patient. We report our experience of the management of patients admitted with liver trauma.

Methods: Retrospective analysis of 75 patients admitted with liver trauma over a 9-year period (1992–2001). Fifty-eight were men (median age 25 years; range 1–76 years). Injury severity was determined using the hepatic injury scale (HIS).

Results: Sixty-three patients were referred from other hospitals. Seventy-two patients suffered blunt trauma. Six patients were classified as grade 5 on the HIS, 43 as grade 4, 17 as grade 3, 8 as grade 2 and 1 as grade 1. Fifteen patients underwent surgery at the referring hospital, which involved perihepatic packing in 80 per cent. Of the 48 patients treated conservatively at the referring hospital, 43 were successfully treated with further conservative management. In total 23 patients required laparotomy in our unit, 18 for liver related injuries. Procedures included 3 hepatic resections and 13 for hepatorrhaphy. Median blood requirement in our unit was 2 units. Four patients developed definite biliary complications treated with endoscopic stenting in three cases and percutaneous drainage in one. A further five intra-abdominal collections required percutaneous drainage. The mortality rate was 8 per cent.

Conclusion: The majority of patients with liver trauma can be successfully treated with conservative management. In unstable patients initial surgical packing and transfer to a specialist unit is recommended.

General Papers 14

Efficacy and durability of botulinum toxin for hyperhidrosis evaluated by patient initiated re-attendance

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Aims: Hyperhidrosis substantially impairs quality of life. Sympathectomy is often ineffective for axillary hyperhidrosis and carries a significant risk of compensatory hyperhidrosis. Botulinum toxin injection offers a temporary but effective treatment. The aim of this study was to assess the value to patients of this treatment as judged by their voluntary, self-initiated re-attendance for further treatment.

Methods: A prospective database of all patients attending for Botulinum toxin injections for hyperhidrosis has been kept since October 1997. After treatment no routine review is arranged. Patients who want further injections must self refer to the clinic when they think it is necessary.

Results: From October 1997 to January 2002 60 patients have received Botulinum toxin for hyperhidrosis (54 axillary, 6 plantar). 45 received their first injections more than 12 months ago and of these 31 (69 per cent) have initiated re-attendance for further treatment at a median of 10 months (IQR 7–13 months) after initial treatment.

Conclusions: Botulinum toxin injection for hyperhidrosis is of sufficient value to at least 69 per cent of patients that they self-initiate repeated therapy. The median duration of efficacy is 10 months.

General Papers 15

The surgical risk scale as an improved tool for risk adjusted analysis in comparative surgical audit

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Aims: Comparative surgical audit is increasingly important, although fraught due to the difficulty of risk adjusted analysis. The surgical risk score was developed to provide surgeon-independent risk-adjusted mortality in general surgery using readily available clinical indices (CEPOD grade of operative urgency, BUPA grade of operative magnitude and ASA grade of patient comorbidities).

Methods: Prospective data collection from 4308 patients admitted under the care of three general surgeons (May 1997–October 1999) created an initial data set of 3144 procedures with 134 deaths. Each procedure was scored by summation of linear scales allocated to CEPOD, BUPA and ASA grades to devise a surgical risk scale (SRS) ranging from 3 to 14. Multi-variate logistic regression analysis involving the three variables and uni-variate analysis of the SRS scores were undertaken. Receiver operating characteristic and calibration curves were formulated. This process was validated on another data set (2780 patients) derived from all admissions to the same surgeons (November 1999–December 2000).

Results: Uni-variate logistic analysis of the SRS score revealed it to be significantly predictive of death ($\beta = 0.84$, $P < 0.001$). The SRS score did not over predict mortality for low risk procedures. The areas under the receiver operating characteristic curve was 0.931 for the initial data set and 0.951 for the validation set.

Conclusion: The SRS is easy to use, formulate and interpret. It has been validated as a tool for risk-adjusted comparative audit in a population of general surgical patients.

General Papers 16

Stored blood products stimulate cancer growth

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Aims: Blood transfusion has often been shown to reduce cancer survival, however, the mechanism of this effect is unknown. We have measured anti-angiogenic agent endostatin, the proangiogenic growth factors, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) in various blood products and assessed their effect upon tumour and endothelial cell growth to attempt to explain this clinical finding.

Methods: Blood products as supplied by the blood bank were maintained at 4 C and every 72 h was centrifuged at 3000 r.p.m. for 15 min, the supernatant underwent measurement of endostatin, bFGF and VEGF by ELISAs. A standard MTS assay measured human umbilical vein endothelial cells (HUVECS) and colorectal cancer cell (Caco-2) proliferation from incubated stored blood supernatant compared to control and cancer patients plasma.

Results: Endostatin in packed red blood cells (2100 pg/mL ± 810) became undetectable within one week of storage, during which time platelets increased bFGF levels by fourfold (4.4 ± 1.9 *versus* 17.4 ± 4.5 pg/mL, $P < 0.001$). Plasma from whole blood contained significantly more VEGF (73.6 pg/mL ± 5.0) than leucodepleted packed cells (22.6 pg/mL ± 1.2, $P < 0.01$). HUVECS and Caco-2 cells increased proliferation upon incubation with stored blood supernatant compared to patient plasma ($P < 0.05$); an effect inhibited by anti-VEGF antibody ($P < 0.05$).

Conclusions: During storage, blood products become proangiogenic stimulating both cancer cells and endothelial proliferation *in vitro*. These findings may have clinical significance in terms of the detrimental effects blood products contribute to cancer patients survival.

General Papers 17

Extended thromboprophylaxis in general surgery reduces the risk of thromboembolism: a meta-analysis

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Aims: Major general surgical operations, especially for malignancy, are associated with a high risk of venous thrombo-embolic (VTE) complications, of which about 40 per cent occur after discharge. In this systematic review we examine the value of extended thrombo-prophylaxis.

Methods: Three randomized, controlled trials, which studied 1 week *versus* 4 weeks of thromboprophylaxis (TP) with low molecular weight heparin (LMWH), were included in the analysis. We used RevMan (Version 4.1.1, Oxford & England 2000) to perform the meta-analysis using fixed odds ratio (OR) to compare groups. All patients undergoing venography were considered and only objectively confirmed VTE events were included in the efficacy analysis. All patients randomized to prolonged prophylaxis and who received a dose of study drug were considered in the safety analysis.

Results: A total of 683 patients were randomized to receive LMWH for 1 week (333 patients) or 4 weeks (350 patients). Total VTE events occurred in 16 (4.8 per cent) patients in the extended group and 40 (11.4 per cent) in the control group (OR = 0.39, 95 per cent CI 0.21–0.71; $P = 0.002$). The risk of bleeding was 3.5 per cent in both groups (OR 0.99, 95 per cent CI 0.47–2.06; $P = 1$).

Conclusions: Extended TP results in significant reduction of the risk of developing VTE in patients undergoing major general surgical operations without any increase in the risk of bleeding.

General Papers 18

Surgery and acute normovolaemic haemodilution (ANH) both impair coagulation

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Aims: To assess the effects of surgery and ANH on coagulation.

Methods: A total of 33 patients randomized to ANH ($n = 17$)/no ANH ($n = 16$), undergoing major gastrointestinal surgery, were prospectively studied. Coagulation tests included aPTTR, INR, platelets, fibrinogen and thrombo-elastography (TEG).

Results: There was no significant difference between groups at entry. In 'no ANH', as operation progressed, there was a decrease in platelet count ($P < 0.001$) and fibrinogen ($P = 0.001$), and increase in aPTTR ($P = 0.002$) and INR ($P = 0.001$). The TEG confirmed this, decrease in alpha angle ($P = 0.006$), MA ($P = 0.003$) and G ($P = 0.001$), increase in K ($P = 0.008$). Prior to knife to skin, ANH reduced platelet count ($P = 0.003$) and fibrinogen ($P = 0.001$), and increased aPTTR (0.003) and INR ($P = 0.001$). R time was shortened ($P = 0.001$) but MA ($P = 0.001$) and G ($P < 0.001$) decreased. Re-transfusion of autologous blood increased platelet count ($P = 0.03$) and fibrinogen ($P = 0.004$), decreased aPTTR ($P = 0.017$) and INR ($P = 0.002$), without affecting the TEG. Between the groups, at the time of knife to skin, the ANH group had a lower platelet count ($P < 0.001$), increased INR ($P = 0.006$), reduced MA ($P = 0.010$) and G ($P = 0.011$), but shortened R time ($P = 0.004$). At 1 h into operation, the platelet count remained lower ($P = 0.002$), the INR ($P = 0.005$) and aPTTR ($P = 0.008$) significantly prolonged. Immediately prior to re-transfusion the only difference was a prolonged INR ($P = 0.023$). By the end of the operation there was no difference between groups. In addition there was no difference in estimated blood loss.

Conclusions: Coagulation is significantly impaired as operation progresses. Acute normovolaemic haemodilution impairs coagulation; this remains significantly impaired throughout operation.

ASGBI: Vascular 01–12

Wednesday, 22 May 14.30–16.30

Vascular 01

Adequate vascular training opportunities can be provided without compromising patient care

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Aims: To review the results of index operations in vascular surgery and to evaluate the impact of a specialist vascular training programme on patient outcome.

Methods: We undertook a 5-year (January 1995–December 1999) review of the weekly collected mortality and morbidity data. The total number, 30-day mortality and stroke rate of all index operations, i.e. AAA repairs (ruptured and elective), CEA and infra-inguinal bypasses (above and below knee, elective and emergency) were recorded. The number of operations performed by trainees under supervision was recorded.

Results: See Tables 1 and 2.

Table 1

Operation	Total number	Trainee (%)	Consultant supervision (%)	Death (%)	Stroke rate (%)
Ruptered AAA	214	186 (86.7)	107 (57.7)	112 (52.3)	N/A
Elective AAA	241	189 (78.4)	159 (84.3)	8 (3.3)	N/A
CEA	240	140 (58.4)	140 (100)	4 (1.7)	14 (5.8)
Infra-inguinal bypass	296	223 (75.5)	201 (90.3)	25 (8.4)	N/A

Table 2

	Death				
	Ruptured aneurysm	Elective aneurysm	CEA	Infra-inguinal bypass	Stroke following CEA
Overall	112/214 (52%)	8/241 (3%)	4/240 (2%)	25/296 (8%)	14/296 (6%)
Performed by consultant	13/28 (46%) (95% CI: 33–71)	1/52 (2%) (95% CI: 0–7)	2/100 (2%) (95% CI: 1–4)	5/73 (7%) (95% CI: 1–14)	8/100 (8%) (95% CI: 3–11)
Trainee consultant assisted	59/107 (55%) (95% CI: 55–62)[†]	5/159 (3%) (95% CI: 1–6)[†]	2/140 (1%) (95% CI: 1–4)[†]	18/201 (8%) (95% CI: 5–12)[†]	6/140 (4%) (95% CI: 2–9)[†]
Trainee not assisted by consultant	40/79 (51%) (95% CI: 41–63)[†]	2/30 (7%) (95% CI: 6–12)[†]			2/22 (9%) (95% CI: 3–20)[†]

Overall vascular trainees performed 74.5 per cent of the index operations of which 82.2 per cent were supervised.

Conclusions: The mortality and morbidity of index operations in our unit compared well with accepted best mortality and morbidity figures. Our unit provides good training opportunities for vascular trainees whilst maintaining satisfactory standards of patient care.

Vascular 02

The implications of changes in vascular practice on higher surgical training

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Aims: To assess the changes in vascular surgical practice over a decade and its implications for higher surgical training.

Methods: Between 1992 and 2001, data for all arterial operations performed under a single vascular surgeon were entered consecutively into a computerized database. The number of vascular index procedures performed per annum was calculated. The index procedures used were: abdominal aortic aneurysm repair, carotid endarterectomy and infrapopliteal reconstruction.

Results: The median number of arterial procedures performed per annum was 238 (range 208–304). Over the decade, there was a steady increase in abdominal aortic aneurysm (AAA) surgery from 53 cases (25 per cent of total arterial workload) in 1992 to 81 cases (33 per cent) in 2001. This was similar for carotid surgery, which increased from 24 cases (11 per cent) in 1992 to 54 cases (22 per cent) in 2001. Higher surgical trainees performed an average of 22 AAA repairs and 15 carotid endarterectomies per annum. In contrast, infrapopliteal reconstruction decreased from 30 cases (14 per cent) in 1992 to 6 cases (2.4 per cent) in 2001. This change in practice has been particularly noticeable in the last 2 years when higher surgical trainees have only performed an average of two infrapopliteal bypasses per annum.

Conclusion: The changes in vascular surgical practice may mean that higher surgical trainees will not receive adequate exposure to infrapopliteal bypass with the current length of training.

Vascular 03

Vascular surgery, CCU and HDU: a 10-year observational study

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Aims: Over the course of the past decade, numerous changes have occurred in the management of patients undergoing vascular surgery operations. The introduction of high dependency units (HDUs) has meant that many patients previously requiring observation in intensive care units (ICUs) are now managed in this new environment. This study reports the chronological changes in resource utilization of patients undergoing major vascular surgery at a district general hospital (DGH).

Methods: Details of all patients admitted to either the ICU or HDU under the care of a single vascular surgeon during the period 1991–2000 were extracted from the prospectively maintained departmental database. Details of the age and sex of patients were obtained together with source of admission, place of discharge and duration of stay. Operative details for each patient were extracted from a prospectively maintained vascular surgery database. Details of patients not attending either HDU or ICU during the postoperative phase were obtained from the same source.

Results: During the 10-year period under study, numerous changes in vascular surgery practice were observed. The HDU unit was opened in 1992.

	ICU			HDU			ICU/HDU				
Year	All	Vasc	Vasc %	All	Vasc	Vasc	Vasc %	HDU (%)	ICU (%)	Vasc %	All
1991	90	44	49				90	44	49	0	100
1992	108	51	47	65	27	42	173	78	45	35	65
1993	91	59	65	43	19	44	134	78	58	24	76
1994	70	32	48	65	31	48	135	63	47	49	51
1995	55	27	49	87	49	56	142	76	54	64	36
1996	85	52	61	43	32	74	128	84	65	38	62
1997	63	37	59	53	29	55	116	66	57	44	56
1998	70	45	64	45	41	91	115	86	75	48	52
1999	22	13	59	68	54	79	90	67	74	81	19
2000	32	12	38	85	63	74	117	75	64	84	16

Conclusions: Subspecialization over the past decade has meant a significantly increased major vascular workload. Since the introduction of the HDU, there has been a significant fall in the use of ICU facilities for routine cases. These changes in resource utilization have significant implications in terms of budget allocation. It would appear that finances, in relation to vascular surgery, should be concentrated on expanding HDU facilities and ensuring vascular surgery expertise amongst ward nursing staff.

Vascular 04

Split-site vascular surgery: twin peaks or double trouble? Implications for patients and trainees

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Aims: To determine whether performing vascular surgery at both the regional vascular centre (RVC) and a district general hospital (DGH) is associated with different clinical outcomes, and how such a practice affects the experience of the trainees at both sites.

Methods: Over a 22-month period, we compared the postoperative mortality of 32 elective and emergency abdominal aortic aneurysm (AAA) repairs, the limb salvage rates following 63 surgical reconstructions for severe leg ischaemia, and the nature of first assistants during 122 arterial procedures, all performed by one consultant in either the RVC or a DGH.

Results: No patient died following elective AAA surgery, but the operative mortality following rupture was higher in the DGH (80 per cent *versus* 14 per cent; ns). Graft patency (84 per cent *versus* 81 per cent; ns) and salvage rates (88 per cent *versus* 83 per cent; ns) 3 months following lower-limb reconstruction were similar in the DGH and RVC. All but one of the cases in the RVC were attended by dedicated level II vascular trainees who were far more likely to perform an anastomosis than the nondedicated level I trainees in the DGH (65 per cent *versus* 18 per cent; $P < 0.01$).

Conclusions: Split-site elective vascular surgery can be performed safely, but there is a conflict between exposure of level I trainees in the DGH to arterial reconstruction and increasing the often limited experience of level II trainees in the RVC.

Vascular 05

Doppler ultrasound in the investigation of thoracic outlet syndrome

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Aims: The diagnosis of thoracic outlet syndrome rests primarily on a clinical evaluation. Doppler ultrasound may be employed to investigate any vascular component. Normative data in this area have not been well established.

Methods: A total of 25 asymptomatic healthy individuals were examined clinically and with ultrasound. The presence of a subclavian bruit was noted. A single operator in turn imaged each arm. The patient was examined in three provocative arm positions: 90° abduction, 90° abduction with external rotation, and an elbow braced back, Roos-type position. Colour images and Doppler spectra were obtained. The duplex was considered positive if a significant alteration in the Doppler spectrum occurred, e.g. at least to monophasic flow. A single radiologist verified all scans.

Results: Eleven female and 14 male subjects were examined, median age 27 years (20–63). Eleven (44 per cent) subjects had an arterial bruit, of which four were bilateral. Sixteen (64 per cent) subjects had a positive arterial duplex, of which four were bilateral. Venous entrapment was detected in eight arms. If for the purposes of this study, ultrasound is considered, the gold standard for the detection of arterial entrapment, the sensitivity of a bruit in detecting such entrapment is 46 per cent, and its specificity 88 per cent.

Conclusions: Given the high prevalence of abnormal Doppler ultrasound findings in an asymptomatic population, duplex may have only a limited role in the investigation of TOCS. Although not a sensitive test, the detection of an arterial bruit correlates well with flow abnormalities appreciable on ultrasound examination. Clinical evaluation should continue to be paramount in the management of TOCS.

Vascular 06

Superficial venous surgery in chronic venous ulceration: the influence on anatomic and haemodynamic function

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Aims: To assess the effects of superficial venous surgery on anatomic and haemodynamic function in legs with chronic venous ulceration.

Methods: Ulcerated legs with an ABPI > 0.85 undergoing surgical correction of superficial venous reflux were studied. Colour duplex imaging and digital photoplethysmography were performed before and 6 weeks following surgery. Legs were stratified into three groups: (A) isolated superficial reflux ($n = 44$), (B) superficial and segmental deep venous reflux ($n = 23$), and (C) superficial and total deep venous reflux ($n = 12$).

Results: Seventy-nine limbs with chronic venous ulceration were studied. Superficial venous surgery abolished segmental deep reflux in 9 of the 23 legs (group B) but only abolished total deep reflux in 1 of the 12 legs (group C) ($P = 0.03$, Chi-square test). Surgery improved median (range) venous refill time from 10 (4–48) to 21 (9–48) s in group A, 11 (4–38) to 16 (6–36) s in group B, and 9 (3–24) to 11 (6–32) s in group C ($P < 0.001$, $P = 0.06$ and $P = 0.08$, respectively).

Conclusions: Superficial venous surgery may correct segmental deep venous reflux, but has little effect on total deep reflux. Superficial venous surgery improves venous haemodynamic function in ulcerated limbs with isolated superficial reflux, and may improve haemodynamic function in legs with mixed superficial and segmental deep reflux.

Vascular 07

Chronic venous ulceration: effectiveness of sapheno-femoral disconnection alone or with long saphenous vein stripping

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Aims: Elderly patients with chronic venous ulceration may benefit from venous surgery, but often wish to avoid general anaesthesia. The aim of this study was to compare the effectiveness of sapheno-femoral disconnection (SFJD) under local anaesthesia and SFJD plus stripping of the long saphenous vein under general anaesthesia in legs with chronic venous ulceration.

Methods: Patients with chronic venous ulceration were assessed with colour duplex imaging to identify those with sapheno-femoral junction reflux. Patients underwent either SFJD alone under local anaesthetic or SFJD with stripping under general anaesthetic. Twenty-four-week ulcer healing rates and 12-month ulcer recurrence rates were compared.

Results: The median (range) age of patients undergoing SFJD alone was 81 (46–90) years ($n = 30$) and of patients undergoing SFJD plus stripping was 68 (31–86) years ($n = 83$). Age-adjusted 24-week ulcer healing rate was 62 per cent for both groups. Twelve-month ulcer recurrence rates were 14 and 12 per cent, respectively ($P = 0.39$, Kaplan–Meier survival analysis).

Conclusions: SFJD plus stripping has no additional short-term benefit over SFJD alone in legs with chronic venous ulceration.

Vascular 08

Hyperhomocysteinaemia, a risk factor for aneurysmal vascular disease?

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Aim: Homocysteine (HCY) is an independent risk factor for occlusive vascular disease, the relationship of hyperhomocysteinaemia with aneurysms has not been established. The aim of this study was to assess the incidence of elevated HCY in patients with aortic aneurysms compared to controls, and patients with occlusive arterial disease.

Methods: We measured HCY levels in 200 patients, 43 age- and sex-matched patients without vascular disease, 97 with aortic aneurysms (64 thoracoabdominal and 33 infrarenal) investigated for surgical intervention, and 60 patients with limb ischaemia (35 claudicants and 25 with critical limb ischaemia). Fasting blood samples were taken; serum separated and stored at −70 °C, HCY levels were measured using fluorescence polarization immunoassay (Abbott $\text{IMx}^{\circledR}$ assay). Results are presented as mean ± SEM with 95 per cent confidence intervals, and the Mann–Whitney *U*-test was used for statistical analysis.

Results: Nine per cent of controls were hyperhomocysteineaemic ($>15\ \mu\text{M L}^{-1}$); the mean HCY level was 9.35 ± 0.64 (95 per cent CI: 8.06–10.65) $\mu\text{M L}^{-1}$. Thirty-two per cent of the patients with aneurysms had elevated homocysteine, 13.20 ± 0.62 (95 per cent CI: 11.97–14.43) $\mu\text{M L}^{-1}$, and 26 per cent of patients with limb ischaemia were hyperhomocysteinaemic 13.24 ± 0.68 (95 per cent CI: 11.88–14.61) $\mu\text{M L}^{-1}$. HCY levels in both aneurysmal and occlusive disease were significantly higher than controls ($P < 0.0001$), and there was no difference between the occlusive and aneurysmal group.

Conclusion: Homocysteine is prevalent in both aneurysmal and occlusive vascular disease. The treatment of hyperhomocysteinaemia is cheap, simple and inexpensive, and may represent a mechanism to modify the disease process.

Vascular 09

Selection of patients for repair of a ruptured aortic aneurysm

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Aims: The aim was to see if the POSSUM and Hardman scoring systems were predictive of outcome after emergency abdominal aortic aneurysm surgery and to see if they could be used in patient selection.

Methods: From 1990 to 2001, 246 patients presented as an emergency with a ruptured abdominal aortic aneurysm (AAA), 41 were treated conservatively. The case notes of these patients were reviewed retrospectively (it was not possible to retrieve four patients' notes), P-POSSUM and Hardman scores were calculated and outcome was recorded.

Results: Of the 205 patients who had surgery, 105 (51 per cent) died. P-POSSUM risk bands and Hardman scores were significantly associated with increased mortality ($P < 0.001$, Chi-square for trend), P-POSSUM was more discriminating, identifying a greater number of high-risk patients.

P-POSSUM-predicted mortality (%)	0–10	10–25	25–60	60–90	90+
Observed operative mortality (%)	0/7	5/30 (17)	34/84 (40)	30/43 (70)	33/37 (89)
Treated conservatively ($n = 41$)	1	0	4	19	17
Hardman score	0	1	2	3	
Observed operative mortality (%)	21/75 (28)	41/76 (54)	27/39 (69)	13/14 (93)	
Treated conservatively ($n = 41$)	0	16	8	17	

Conclusions: POSSUM identified more high-risk patients whilst Hardman scoring although identifying fewer high-risk patients was much simpler to perform. Risk scoring systems may have a fundamental role in helping to identify patients with a ruptured AAA in whom surgery is futile.

Vascular 10

Simvastatin preserves elastin and reduces the inflammatory response in aortic aneurysmal tissue

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Aims: The loss of elastin and subsequent weakening of the aneurysmal aortic wall is mediated by high levels of matrix metalloproteases and inflammatory cytokines. HMG CoA reductase inhibitors ('statins') may offer a pharmacotherapeutic strategy to prevent aneurysm expansion due to inhibition of both MMP formation and cytokine secretion.

Methods: Elastin degradation was examined in a porcine model of aneurysmal disease. Sections of 1 cm^2 of healthy porcine aorta were treated with elastase for 24 h to initiate an inflammatory and proteolytic response. Following organ culture with incremental doses of simvastatin for 14 days, tissue was harvested for histological examination and MMP activity. The inflammatory response within aortic tissue was examined using full thickness human aneurysmal explants cultured in simvastatin for 48 h. Cytokine production was measured in the conditioned medium by ELISA, and MMP activity quantified by substrate gel zymography.

Results: Addition of simvastatin to the culture medium resulted in preservation of elastin and reduction in the levels of MMPs and interleukin-6.

		Simvastatin			*P*-value
	Control	0.1 mM	0.3 mM	1 mM	(ANOVA)
Elastin (%)	22	24	40	41	<0.001*
MMP-9 (o.d.)	0.93	0.61	0.42	0.35	0.0035*
IL-6 (ng mL^{-1})	979	594	428	N/A	0.04*
MMP-2 (o.d.)	0.182	0.114	0.116	0.112	0.03*

Conclusions: Simvastatin has beneficial effects on the inflammatory and proteolytic processes within the aneurysmal aortic wall, and has the potential to retard aneurysm expansion in clinical trials.

Vascular 11

Management of popliteal aneurysms

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Aims: The best management of patients with either thrombosed or nonthrombosed popliteal aneurysms has yet to be established. This paper describes our experience managing such aneurysms.

Methods: A prospective study was carried out of all patients presenting with popliteal aneurysm (PA) during January 1988–December 2001. Since 1993, asymptomatic PA <3 cm in diameter without distortion were managed conservatively. Ultrasound scans were carried out at 6-monthly intervals. These results were compared with conservative management of popliteal aneurysms of >3 cm in diameter in patients who declined or were unfit for operation and with those patients who underwent elective bypass of PA. Results of patients presenting with thrombosed PA are also described.

Results: Fifty-eight patients (two women) presented with 92 PA. Some 39 had thrombosed PA and these patients were significantly more likely to have bilateral aneurysms ($P < 0.001$). Of the non-thrombosed PA managed conservatively none below 3 cm in diameter thrombosed. Patency of PA 2–3 cm in diameter was no worse than patency following elective bypass ($P = 0.37$). The risk of postoperative complications was greater with thrombosed compared with non-thrombosed PA ($P < 0.005$). Pre-operative lysis for thrombosed PA was associated with more complications than operation and on table lysis ($P < 0.05$).

Conclusions: Careful monitoring of asymptomatic PA < 3 cm in diameter is safe. Pre-operative lysis is associated with increased risks compared with operation alone in those patients presenting with thrombosed PA.

Vascular 12

Femorodistal bypass: an index operation?

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Aims: The three index operations for vascular training comprise abdominal aortic aneurysm repair, carotid artery endarterectomy and femorodistal bypass graft operations. We feel that during the last decade, the number of primary femorodistal bypass operations performed has continued to decline in institutions where percutaneous transluminal angioplasty (PTA) has become the primary treatment for critical limb ischaemia (CLI).

Methods: A retrospective study of the management of chronic CLI in our institution over a 6-year period (1994–1999). Patients were grouped according to the date of first presentation into three groups: group 1 presented between 1994 and 1995, group 2 between 1996 and 1997 and group 3 between 1998 and 1999.

Results:

- Group 1 comprised 185 patients with 222 ischaemic limbs, of those 19 primary crural lesions were treated (6 by PTA alone and 13 by PTA and/or surgery).
- Group 2 comprised 180 patients with 204 ischaemic limbs, of those 29 primary crural lesions were treated (16 by PTA alone and 13 by PTA and/or surgery).
- Group 3 comprised 161 patients with 182 ischaemic limbs, of those 24 primary crural lesions were treated (13 by PTA alone and 11 by PTA and/or surgery).

Conclusions: PTA is becoming the primary choice for the treatment of CLI including distal lesions, and primary femorodistal bypass graft procedures are declining (six–eight per year). We question the rationale of including such an uncommon operation into the vascular training index procedures.

ASGBI: Upper GI 01–12

Wednesday, 22 May 14.30–16.30

Upper GI 01

Prospective randomized trial of proton pump inhibition, prokinesis or combination for bile reflux

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Aims: This prospective, randomized trial assessed the effect of omeprazole, cisapride, or a combination, on the bile acid component of refluxate, aspirated as precisely time-stamped samples, from patients experiencing proven gastro-oesophageal reflux.

Methods: Thirty-eight patients (median age 55, 30 males) were randomized to receive cisapride 20 mg bd, omeprazole 20 mg od or both. Thirty-four completed the trial. After endoscopy and manometry, pH monitoring with oesophageal aspiration studies were performed before and after 4 weeks of medication. Symptoms, pH traces and aspiration volumes were compared. Pre- and post-drug aspiration samples (1294 in all) were sent for high performance liquid chromatography (HPLC). Concentrations of, and number of samples containing, bile acids were compared.

Results: Median [total bile acid] is calculated by summing the concentrations of 15 separate bile acids (in μM/L) identified at HPLC in each sample and taking the median for each patient.

	Pre- or post drugs	Cisapride	Omeprazole	Combination
No. patients (samples)	Pre	11 (211)	11 (220)	12 (250)
	Post	11 (181)	11 (193)	12 (239)
De Meester & Johnson score	Pre	62.2*	18.9**	24.95***
	Post	20.05*	5.7**	5.9***
Median [total bile acid]	Pre	15.0†	21.0††	41.5†††
	Post	2.5†	5.0††	1.5†††
No. samples containing bile acid	Pre	119‡	135‡‡	165‡‡‡
	Post	101‡	109‡‡	91‡‡‡

$^{*}P = 0.26$, $^{**}P = 0.005$, $^{***}P = 0.008$. Wilcoxon. †$P < 0.04$, ††$P = 0.17$, †††$P = 0.003$. Wilcoxon. ‡$P > 0.9$, ‡‡$P > 0.3$, ‡‡‡$P < 0.001$. Chi-squared. Pre-treatment: * *versus* ** *versus* *** ns. Kruskal–Wallis. Post-treatment: * *versus* **, $P = 0.031$; * *versus* ***, $P = 0.012$; ** *versus* *** ns. Mann–Whitney.

Conclusion: Cisapride reduced bile acid concentrations but not episodes of reflux; omeprazole reduced neither. Combination therapy is better than either individual drug, with significant reductions in both.

Upper GI 02

Outcome after surgical management of duodenal polyposis associated with familial adenomatous polyposis

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Aim: Patients with severe (Spigelman IV) duodenal polyposis associated with familial adenomatous polyposis (FAP) followed up at St Mark's Hospital have been shown to be at high risk of malignant transformation. A retrospective review of such patients who underwent prophylactic resection was undertaken in order to ascertain outcome and form the largest series in the literature.

Methods: The outcomes of 14 consecutive patients, median age 55 (range 40–70) years, with severe duodenal polyposis associated with FAP underwent resection between 1994 and 2001. Their preoperative assessment by routine endoscopy, biopsy, endoscopic ultrasound and CT scan, management and histology was reviewed.

Results: There were 13 pancreatoduodenectomies and one local resection; three patients developed major postoperative complications, one of whom required reoperation for secondary haemorrhage, and there was one death within 60 days due to a pulmonary embolus. Four patients had carcinomas, two of which were not detected preoperatively and three of whom died of recurrence (9–36 months postoperatively); the remaining 10 patients are alive and disease free at a median of 17 (range 4–77) months. Preoperative investigation had difficulty in delineating the progression to invasion.

Conclusion: Patients with severe duodenal polyposis associated with FAP should be counselled with regard to prophylactic resection given the high rate of malignant transformation. Pancreatoduodenectomy, whilst associated with significant morbidity, is the only procedure that ensures complete removal of polyp-bearing duodenum and occult malignancy. Malignant transformation is associated with a poor prognosis.

Upper GI 03

Ampullary neoplasms: presentation, outcome and prognostic factors

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Aims: The purpose of this study was to analyse the outcome and determine the predictors of survival for ampullary neoplasms.

Methods: Over an 11-year period, 583 patients were treated for peri-ampullary tumours, 88 of whom had a histologically proven ampullary neoplasm. Prospectively gathered data were analysed to assess predictors of survival, using Kaplan–Meier, t-test and Cox regression.

Results: At presentation, five patients (6 per cent) were unresectable. Of those patients operated ($n = 83$; 94 per cent), 81 were resected (resection rate 92 per cent), with no operative mortality. Pathological examination of the specimen showed benign tumor ($n = 10$; 12 per cent), carcinoma in situ ($n = 1$; 1 per cent) and invasive cancer ($n = 70$; 86 per cent). Median survival was 45.8 months for resectable patients *versus* 8.0 months for unresectable patients ($P < 0.0001$). On univariate analysis, age <70 ($P = 0.02$) and bilirubin <75 mmol/L ($P = 0.04$) favoured long-term survival. Of the 70 resected cancers, factors associated with a significantly ($P < 0.05$) worse long-term survival on univariate analysis included poorly differentiated histology, invasion of the pancreas (T3), positive nodes, and perineural invasion. Age, sex, presence of jaundice and type of procedure had no influence on survival. On multivariate analysis, tumour differentiation, perineural invasion and bilirubin level were independently related to survival.

Conclusion: An aggressive surgical approach for ampullary tumours is justified by the low proportion of benign lesions, the absence of operative mortality and the improved long-term survival. Resectability, tumour differentiation, absence of perineural invasion and bilirubin level appear to best predict long-term survival.

Upper GI 04

A postoperative prognostic nomogram for patients undergoing resection for adenocarcinoma of the pancreas: assessing the individual's risk of recurrence

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Introduction: Management of resected pancreatic adenocarcinoma remains controversial with recent randomized trials suggesting a role for adjuvant therapies. Patient selection for adjuvant therapy is currently based on models that define discrete risk groups for recurrence. However, no information exists which combines clinicopathological factors to predict an individual's probability of disease recurrence.

Aim: The aim was to develop a prognostic model for disease-specific survival (DSS) following resection using clinicopathological parameters.

Methods: A prospective database established in 1983 was used to identify patients with pancreatic adenocarcinoma resected between October 1993 and April 2000. Clinicopathological/operative details were recorded. Follow-up was obtained as of October 2001. By using an accelerated failure time regression model, a nomogram was developed to predict median and 3-year DSS. The nomogram was validated internally using bootstrapping.

Results: A total of 546 patients underwent resection for pancreatic adenocarcinoma and included. Sex distribution was equal with the pancreatic head being the predominant site of disease ($n = 492$, 90 per cent). The median DSS of the entire group was 17 months (95 per cent CI 15.6–18.7 months). In multivariate analysis, size, differentiation, number of positive nodes, margin status, and concomitant splenectomy were significant survival variables ($P < 0.05$) and included in the nomogram. Calibration plots suggested that the nomogram was well calibrated for all predictions.

Conclusions: This nomogram predicts better than chance and discriminates better than simply providing all patients with an average survival estimate ($P < 0.05$). It can be used to predict the 3-year DSS for an individual patient following resection. We believe that this nomogram gives novel information allowing for accurate assessment of prognosis, treatment selection, and clinical trial design.

Upper GI 05

Management of acute severe pancreatitis without necrosectomy

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Aim: The standard approach to pancreatitis with necrosis is necrosectomy. Recently only those with infected necrosis were operated upon. An alternative approach is drainage under radiological control.

Methods: An audit by retrospective review of patients with acute pancreatitis managed by the pancreatic firm between January 1996 and December 2000 was undertaken.

Results: A total of 105 patients (M 46, F 59) were admitted, mean age in years 48 (range 17–79); 67 were tertiary referrals (64 per cent). Aetiology was gallstones 41 (40 per cent), alcohol 36 (34 per cent), idiopathic 16 (15 per cent) and other 11 (11 per cent), 25 had oedematous pancreatitis (24 per cent), 11 had complicated pancreatitis without necrosis (10 per cent) and 69 patients had necrosis (66 per cent), 26 <30 per cent (38 per cent), 29 30–50 per cent (42 per cent), 14 >50 per cent necrosis (20 per cent). Organ failure occurred in 50 patients (one 29, two 11, three 3, four 5, five 2). Radiologically guided percutaneous drains were placed in 51 of those with necrosis (74 per cent), 30 of whom were infected (59 per cent). Three patients underwent therapeutic angiography (3 per cent) and 28 had an ERCP (27 per cent). 42 patients (40 per cent) received antibiotics based on either blood and/or drain cultures. Mean length of stay was 41 days, range 1–164, whilst for those with necrosis was 52 days, range 3–164. Sixteen patients were admitted to ITU (15 per cent), mean length of stay 19 days (range 1–70). There were five deaths (5 per cent) all in the necrosis group, four of whom were infected.

Conclusion: A non-operative approach to acute severe pancreatitis gives results similar to these series in which selective necrosectomy is undertaken.

Upper GI 06

A prospective evaluation of exocrine pancreatic function in patients with acute pancreatitis

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Aims: To assess pancreatic exocrine function in patients recovering from first attack of acute pancreatitis, and to evaluate its relationship to severity of attack and the development of local pancreatic complications and endocrine pancreatic insufficiency.

Methods: Between December 2000 and November 2001, 23 patients were prospectively evaluated. Pancreatic exocrine function was measured by the faecal elastase-1 test. Attacks were classified as mild ($n = 16$) or severe ($n = 7$) according to the Atlanta criteria.

Results: Pancreatic exocrine insufficiency was significantly more frequent in patients recovering from severe attacks than mild ($n = 6$, 86 per cent *versus* $n = 2$, 13 per cent, $P = 0.002$), and in those who developed pancreatic necrosis or pseudocyst than those who did not [6 of 7 patients *versus* 2 of 16 patients, and 5 of 5 patients *versus* 3 of 18 patients, respectively; $P = 0.002$]. The development of exocrine insufficiency correlated strongly ($P < 0.001$) with the presence of pancreatic necrosis and pseudocyst formation, but not with pancreatic endocrine insufficiency ($n = 4$, $P = 0.07$). 'Severe' exocrine insufficiency ($n = 7$) occurred in all six patients with pancreatic necrosis involving more than one-third of the gland. Exocrine insufficiency persisted during follow-up (median 3 months) in patients with severe disease.

Conclusions: Pancreatic exocrine insufficiency is a common occurrence in patients recovering from severe acute pancreatitis, and its severity relates to the extent of pancreatic necrosis. A follow-up study is required to evaluate the long-term potential for recovery, and the duration of therapy with pancreatic enzyme supplementation.

Upper GI 07

Cryotherapeutic ablation for liver tumours – experience of a United Kingdom series

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Aims: Over the last decade cryotherapy has been reported to improve survival for patients with hepatic metastases. We compared our 5-year experience of cryoablative techniques for colorectal (CR) and non-colorectal (Non-CR) metastases.

Methods: Data were analysed retrospectively from two centres in the North–West of England. Survival analyses were calculated using the Kaplan–Meier method.

Results: Over a 7-year period (1993–2000), 57 patients underwent cryotherapy for malignant hepatic tumours. There were 36 males (63.2 per cent), and 21 females (36.8 per cent). The age of the patients ranged between 17 and 83 years (mean 60, median 57.8 years). Forty-one patients had CR metastases with between 1 and 11 lesions per patient, ranging in size from 2 to 10 cm. Preoperative carcinoembryonic antigen (CEA) levels (mean = 444.1 µg/L) were significantly reduced following treatment in the CR patients with a mean postoperative level of 6.22 µg/L ($P = 0.002$, McNemar test). Two patients developed cryoshock and four other patients suffered cardiorespiratory complications. The remaining 16 patients with Non-CR tumours (6 neuroendocrine metastases, 5 hepatocellular carcinomas (HCC), 3 sarcomas, 1 cholangiocarcinoma and 1 insulinoma) all received cryotherapy alone. There were no major complications in this group. The 3- and 5-year survivals for Non-CR and CR metastases were 80 and 32 per cent and 33 and 6 per cent, respectively. Survival for Non-CR metastases was significantly better than for CR metastases ($P = 0.005$, log rank test).

Conclusion: Cryotherapy is an effective form of hepatic ablation. Cryotherapy for noncolorectal metastases has a greater long-term survival benefit with fewer complications when compared to colorectal metastases ($P = 0.005$, log rank test).

Upper GI 08

Hepatic resection for suspected malignancy subsequently shown to be benign pathology

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Introduction: Hepatic resection for selected patients with liver metastases or proximal cholangiocarcinoma confers significant survival advantages

Confirming hepatic or proximal bile duct malignancy preoperatively remains a challenge. The aim of this study was to establish the incidence and outcome of major liver resection in patients with suspected malignancy subsequently shown to have benign pathology.

Methods: Analysis of a prospectively collected data from patients undergoing hepatic resection between 1989 and 2001.

Results: Of 250 consecutive patients undergoing hepatic resection for presumed malignancy, 18 patients (7.2 per cent) (13 females, 5 males; mean age 50 years) had benign pathology. There was no postoperative mortality in this group but four patients (22 per cent) developed postoperative complications.

Presumed pre-operative diagnosis	Total resections	Final Pathology		'False positives'
		Benign	Malignant	
Malignant biliary strictures	41	10	31	24.4%
Hepatocellular carcinoma	34	2	32	5.9%
Gallbladder carcinoma	15	2	13	13.3%
Secondary liver tumours	160	4	156	2.5%
Total	250	18	232	7.2%

Conclusion: Although hepatic resection remains a potentially curative procedure for patients with tumours involving the liver parenchyma or proximal bile ducts, preoperative confirmation of malignancy remains difficult, with a 'false positive' rate of 7.2 per cent. Despite appropriate investigation a subset of patients with benign disease will still be subjected to major hepatic resection which should be undertaken in a specialist unit.

Upper GI 09

Outcome after antireflux surgery – a comparison between open Nissen, laparoscopic Nissen and laparoscopic Watson fundoplications

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Aims: This study compares the outcome of three different techniques of antireflux surgery, carried out at three regional specialist units.

Methods: Patients undergoing open Nissen fundoplication (Newcastle (ON), $n = 76$), laparoscopic Nissen fundoplication (Edinburgh (LN), $n = 119$), and laparoscopic Watson fundoplication (Aberdeen (LW), $n = 254$) between December 1993 and February 2001 were identified from prospectively compiled databases. Patients were sent a questionnaire, including two gastrointestinal symptom scores (DeMeester and Gastrointestinal Symptom Rating Scale (GSRS)).

Results: A total of 357 (80.6 per cent) patients completed questionnaires; no differences in response rate were observed among centres. Median time since surgery was 3.8 years for LW group, 2.4 years for LN group and 3.5 years for ON group ($P < 0.001$). Only a mean of 6.5 per cent patients reported a poor outcome. DeMeester score (GORD symptoms) was highest following LW and lowest following LN ($P = 0.009$), and increased with time from operation. However, logistic regression revealed no difference among the procedures after allowing for the effect of time, suggesting that time since operation is important, not procedure type. Overall, LN patients reported greater inability to belch ($P = 0.007$), however, this symptom also improved over time ($r = -0.14$, $P = 0.009$), and there was no difference among groups after taking time since operation into account. In addition there was no significant difference in the GSRS scores, painful bloating or inability to vomit between groups.

Conclusion: This study has confirmed that the long-term outcome following all three procedures is similar with gradual reduction in inability to belch with time and some recurrence of other GORD symptoms.

Upper GI 10

Prospective evaluation of bone scintigraphy as a staging investigation for oesophageal carcinoma

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Aims: From 1990 to 1999, 9 per cent (11/125) of patients undergoing radical oesophagectomy for node positive carcinoma re-presented with symptomatic bone metastases within 12 months of surgery. The aim of this study was to prospectively evaluate the introduction of preoperative bone scintigraphy.

Methods: From 01 December 2000 to 30 November 2001, 159 patients with oesophageal carcinoma were assessed. Tc^{99}-HDP bone scintigraphy was performed for patients identified as having transmural (T3) disease and lymph node involvement (N1) by conventional investigations (CT and endoscopic ultrasound), and who were otherwise suitable for radical treatment.

Results: A total of 69 patients (43 per cent) were suitable for radical treatment and of these 45 patients (median age 62 years, M:F ratio 3:1) staged with T3N1 disease underwent bone scintigraphy. The histological diagnosis was adenocarcinoma in 76 per cent and squamous carcinoma in 24 per cent. Bone scintigraphy was normal or showed degenerative change in 33 patients (73 per cent), and demonstrated areas of abnormal radioisotope uptake requiring further investigation in 12 patients (27 per cent). In six patients (13 per cent) plain radiographs, MRI, and biopsy confirmed the presence of bone metastases; a single metastasis in five patients (iliac-2, humerus-2, femur-1) and multiple vertebral metastases in one patient. These six patients received palliative treatment. No patient undergoing bone scintigraphy has developed bone metastases following surgery ($n = 35$) or chemoradiotherapy ($n = 4$), after initial median follow-up of 5 months.

Conclusions: Bone scintigraphy is essential to exclude metastatic disease prior to the radical treatment of advanced oesophageal carcinoma. Bone is frequently the first site of distant metastatic spread and these patients are most appropriately managed by nonsurgical palliation.

Upper GI 11

Multivariate analysis of survival following surgery for oesophageal and gastric cancer: results from a population based cohort of 1302 patients

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Aim: To elicit factors associated with survival in unselected patients undergoing oesophageal or gastric cancer resection.

Methods: The population based Scottish Audit of Gastric and Oesophageal Cancer (SAGOC) collected data from every patient diagnosed with oesophageal, junctional or gastric cancer in Scotland over 2 years to September 1999 with 1 year minimum follow-up. Data were analysed by univariate, case-mix-adjusted then multivariate analysis.

Results: For the 3293 patients (1302 of whom underwent surgery) overall survival was 32 per cent at 1 year and 17 per cent at 2 years. For the 1302 (39.5 per cent) surgical patients, survival was significantly better ($P < 0.001$): 54 per cent at 1 year and 33 per cent at 2 years. Factors adversely affecting survival by multivariate analysis in the surgical group (Table) were comorbid disease (ASA 4 or 5) and margin involvement in the resected specimen. A better outcome was associated with a history of peptic ulcer or *H. pylori* infection, nonsmokers or junctional cancer.

Factor	Hazard ratio	Confidence intervals	Probability
ASA grade 4 (178 patients)	HR 1.35	1.00–1.81	$P = 0.047$
ASA grade 5 (20 patients)	HR 2.46	1.42–4.27	$P = 0.001$
Margin involvement	HR 1.97	1.32–2.96	$P = 0.001$
Nonsmoker	HR 0.78	0.64–0.94	$P = 0.01$
Junctional cancer	HR 0.7	0.51–0.95	$P = 0.021$
History of ulcer/Helicobacter	HR 0.79	0.63–0.99	$P = 0.04$

Neither the size of hospital of diagnosis nor the caseload for hospital of surgery had any impact on survival by univariate, case-mix-adjusted or multivariate analysis.
Conclusion: For patients undergoing oesophagogastric surgery, patient fitness, the site of the cancer and the completeness of resection are significantly related to survival.

Upper GI 12

Oesophago-gastric stromal tumours: a single unit experience

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Aims: Gastrointestinal stromal tumours (GIST) represent a rare group of heterogenous mesenchymal tumours. The aim of this study was to analyse the clinical presentation and outcome for patients with oesophago-gastric stromal tumours managed in a single institution.
Methods: Of 1312 patients undergoing assessment for oesophago-gastric tumours from 1994 to 2001, 25 (2 per cent) patients (median age 60 years, M:F ratio 1:1.1) had stromal tumours. The clinical features, management and long-term outcome was evaluated for these patients.
Results: A total of 19 (76 per cent) gastric and 6 (24 per cent) oesophageal stromal tumours were seen during this period. The commonest symptoms were acute ($n = 2$) or chronic ($n = 10$) gastrointestinal haemorrhage (48 per cent), indigestion (20 per cent) and anorexia (32 per cent). Twenty underwent surgical resection (15 gastrectomy, 5 subtotal oesophagectomy). There were no in-hospital deaths. Three patients were unfit for surgery and one patient had an unresectable tumour at laparotomy. Tumours were classified as leiomyoma (80 per cent) and leiomyosarcoma (20 per cent) according to cellular atypia and mitotic rate. Leiomyosarcomas (mean diameter = 8 cm) were larger than leiomyomas (mean diameter = 4.1 cm, $P = 0.03$). 10 (45.5 per cent) tumours were epithelioid in origin including all sarcomas and 12 (54.5 per cent) were spindle cell. At a median follow up of 32 months all patients with a benign diagnosis were alive with no recurrent disease. Three of four sarcomas died of local recurrence at 6, 52 and 66 months.
Conclusion: GISTs of the upper gastrointestinal tract are rare and most commonly present with gastrointestinal haemorrhage. Surgical resection has excellent outcome but tumour size and epithelioid cell origin are associated with an increased risk of malignancy.

British Obesity Surgery Society 01–04

Wednesday, 22 May 17.05–18.15

Obesity 01

Laparoscopic gastric banding: a significant postoperative radiological workload

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Aims: To assess the role and workload of radiology in the postoperative assessment of patients following adjustable laparoscopic gastric band (Lap-Band) insertion. The radiological assessment of band tightness, the technique of band adjustment and the detection of complications are presented.

Methods: The study population included 32 patients who had undergone Lap-Band insertion over a 4-year period. Postoperative assessment involved monitoring the patients' symptoms and weight loss by serial measurement and calculation of body mass index (BMI). All radiological interventions were documented, including contrast swallows and fluoroscopically controlled adjustments by needle access to the subcutaneous port connected to the Lap-Band device. All needle adjustments were performed using bi-plane fluoroscopy and monitored by contrast swallows. The number and nature of any radiologically detected complications were also recorded.

Results: A total of 163 radiological procedures were required in 32 patients. These included 126 needle adjustments (either tightening (injection) or loosening (aspiration) of the band) and 37 contrast swallows, where there was an apparent discrepancy between the patient's symptoms and serial weight loss assessment. Complications included gastric band leakage and erosion into the stomach lumen (three), leakage from the port and connections (two), shifting of the port (five) and rotation of the gastric band (six).

Conclusions: Adjustable gastric band insertion is not free of complications and creates a significant and challenging postoperative workload for radiology. Particular difficulty relates to assessment of band tightness, and radiological expertise is required in needle adjustment of the band and detection of complications.

Obesity 02

Laparoscopic gastric banding for the treatment of morbid obesity: is it worthwhile?

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Aim: Laparoscopic gastric banding (LGB) has recently become an attractive minimally invasive surgical procedure for the treatment of morbid obesity. We report our preliminary experience performed in a single unit.

Methods: From January 1998 to December 2000, 17 patients were admitted for treatment of morbid obesity. Mean age was 42 years (range: 27–52 years). There were 5 males and 12 females. All patients were fully evaluated in a bariatric surgical clinic prior to surgery. Patients were reviewed by the multidisciplinary team at 6 weeks and regularly thereafter. Quality of life was assessed via a postal questionnaire.

Results: There was no operative mortality. LGB was inserted successfully in 13 cases (76 per cent), three patients (18 per cent) were converted into open insertion and the procedure was abandoned in one patient because of technical difficulties. Mean hospital stay was 5 ± 1 days. Complications occurred in seven patients (41 per cent), including port site infection (two patients), slippage (four cases) and gastric erosion in one patient needing removal of the device. Late complications include one patient with GORD and three patients with radiological evidence of oesophageal dilatation. Median follow up was 14 months. Mean excess weight loss (EWL) was 18.8 per cent. Three patients had >50 per cent EWL and/or a postoperational BMI of <35.

Conclusion: LGB has been reported as a safe, effective procedure in the treatment of morbid obesity, however, complications such as port slippage, erosion and oesophageal dilatation may occur. The long-term consequences of oesophageal dilatation are unknown and further band inflation should be done with caution in these patients.

Obesity 03

Laparoscopic obesity surgery – initial experience with a tailored approach

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Aims: To assess the clinical outcomes from a range of laparoscopic antiobesity procedures, tailored individually to morbidly obese patients.

Methods: All patients listed for primary bariatric surgery between March 1999 and January 2002 by either of the two authors were offered a laparoscopic procedure. The choice of procedure was determined primarily by body mass index (BMI) and by patient's choice after counselling. Relevant data were collected prospectively for analysis.

Results: All patients opted for a laparoscopic procedure. There were 64 primary obesity operations. These comprised 26 vertical gastric stapling (±sleeve gastrectomy) (VGS), 8 Roux-en-Y gastric bypass (RYGB) and 30 laparoscopic adjustable gastric bands (LAGB). Median preoperative BMI was 47 (35–73) for VGS, 53 (43–66) for RYGB and 46 (33–58) for LAGB. There was one conversion during RYGB for bleeding. The median duration of operation for VGS, RYGB and LAGB, respectively, were 135, 390 and 90 min. The median postoperative stay was 3, 7 and 2 days for the same groups. Complications requiring reintervention or readmission occurred in seven patients (two staple line leak, one anastomotic stricture, one GI bleed, two band slippages, one gastritis). Weight loss was slowest after LAGB, but similar to equivalent open procedures in our institution for stapling and RYGB.

Conclusions: The full range of primary bariatric procedures available at open surgery can be applied laparoscopically. Laparoscopic RYGB in particular is a challenging procedure with a steep learning curve.

Obesity 04

Laparoscopic reversal of bariatric bypass: indications persist, risk depends on hypoalbuminaemia, not surgical approach

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Background: In the last quarter of the twentieth century, over 100 000 jejunoileal bypasses (JIBP) were performed since the first description by Kermen in 1954. In the mean time, the long-term metabolic risks of this procedure is well known. Although there are more optimistic reports concerning the biliopancreatic bypass (BPBP, Scopinaro), our own experience is otherwise. We continue to see patients with both types of bypass requiring reversal for the same indications. Could these patients be offered the advantages of laparoscopic reversal?

Methods: In the period between April 1996 and April 2001, all 17 patients presenting for reversal were considered for laparoscopy. The technique is presented elsewhere.

Results: There were five planned open reversals, four conversions from laparoscopy to open and eight complete laparoscopic reversals. Of 17 reversals, 12 were for JIBP and 5 for BPBP. In the open group, there were two fatalities and two patients with complicated course. One patient in the laparoscopic group had an anastomotic leak requiring reoperation. All the patients who died or suffered complication had a preoperative plasma albumin below $30\,\mathrm{g\,L^{-1}}$ (median: $21\,\mathrm{g\,L^{-1}}$), whereas all the uncomplicated patients had values greater than $30\,\mathrm{g\,L^{-1}}$ (median: $40\,\mathrm{g\,L^{-1}}$).

Conclusion: These data show that laparoscopic reversal is usually technically feasible. Since most patients require a secondary bariatric procedure, the low potential for adhesions is of value. Furthermore, many patients continue to have an abnormal body image. The statistically significant difference in albumin levels, not the surgical approach, appears to dictate the outcome.

ASGBI: Vascular 13–19

Wednesday, 22 May 17.05–18.15

Vascular 13

Regional versus general anaesthesia for carotid endarterectomy (CEA): impact of change in practice

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Aims: This study compares local anaesthesia (LA) by cervical plexus nerve block with general anaesthesia (GA).

Methods: All patients who underwent CEA from January 1997 to December 2001 were reviewed; LA was used almost exclusively over the last 2 years. Differences in incidence of postoperative death, neurological dysfunction, cranial nerve injury and haematoma, the need for peroperative shunting, and duration of hospital stay were assessed.

Results: A total of 371 CEAs were carried out in 363 patients (237 men, 126 women), 179 under GA and 192 under LA. Indications were transient ischaemic attack (140), stroke with recovery (134), amaurosis fugax (85) and asymptomatic stenosis (12). Peroperative shunting was used in 66 (37 per cent) GA operations and 38 (20 per cent) LA operations ($P < 0.01$). There were five strokes and six transient neurological events; eight (4.7 per cent) patients developed such problems with GA and three (1.6 per cent) with LA ($P < 0.05$). There were four deaths, three (1.7 per cent) after GA and one (0.5 per cent) after LA ($P > 0.5$); all deaths after GA were due to stroke, while that after LA was due to pulmonary embolism. There was no significant difference between the groups in incidence of myocardial infarction, cranial nerve injury and haematoma formation. Median (range) duration of hospital stay was 4.5 (3.0–14.0) days for the GA group and 3.0 (2.0–10.0) days for the LA group ($P < 0.001$).

Conclusion: Employing LA rather than GA for CEA has resulted in a significant reduction in incidence of postoperative stroke and death, need for peroperative shunting and duration of hospital stay. LA offers clinical and cost advantages over GA.

Vascular 14

Do trainees jeopardize the outcome of infra-inguinal bypass?

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Aim: A surgeon's accountability for his outcome figures may detract from realizing training opportunities during major procedures, and whilst patient welfare must take priority, the surgeons of tomorrow require adequate training. This study aimed to validate the participation of trainees in infra-inguinal arterial reconstruction in our institution.

Methods: Interrogation of our audit database covering infra-inguinal bypass surgery during a 5-year period provided details of operations performed by consultants and trainees. Outcome measures were cumulative patency, limb salvage using Kaplan–Meier curves, and 30-day mortality.

Results: Trainees performed 127 (27 per cent) infra-inguinal bypass operations: 51 (40 per cent) above knee; 46 (36 per cent) below knee; 30 (24 per cent) distal. Consultants performed 341 (73 per cent) operations, 133 (39 per cent) above knee, 84 (25 per cent) below knee and 124 (36 per cent) distal. Similar patient demographics were noted between the two groups, and the outcome measures of each group are summarized in the table. Cumulative patency and limb-salvage rates for operations performed by trainees were compared to those by consultants and no statistical differences were found ($P = 0.2601$ and 0.9026, respectively; log-rank test). Thirty-day mortality was significantly higher after operation by a consultant, 5.5 per cent, compared to a trainee, 1.6 per cent ($P < 0.0001$, Chi-square test).

		Cumulative patency % (*n*)		Limb salvage % (*n*)	
	Total	1 year	3 years	1 year	3 years
Trainee	127	76% (73)	64% (16)	67% (65)	51% (12)
Consultant	341	71% (176)	60% (59)	70% (159)	51% (48)

Conclusion: The outcome after infra-inguinal bypass is not jeopardized by a trainee performing the operation, assuming appropriate supervision. The higher mortality in the consultant-operated group probably reflects appropriate case selection, with training allocated to fitter patients.

Vascular 15

Risk stratification of abdominal aortic aneurysm surgery: the solution to HDU case selection?

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Aim: Development of a risk-stratification model for abdominal aortic aneurysm (AAA) surgery for allocation of low-risk cases to high-dependency unit (HDU) care.

Methods: Prospective APACHE II data were collected from 1896 patients undergoing emergency and elective AAA repair admitted to 20 ICU in the Thames region since 1992. The prognostic ability of the APACHE II system was evaluated using postoperative physiological measurements collected peri-ICU admission. New prognostic models for inhospital mortality were developed using multilevel logistic regression (MLR) and logistic regression (LR) optimized by bootstrap re-sampling (10 000 iterations). Four independent risk factors were identified: emergency operation, chronic health status, age and the acute physiology score. Multilevel modelling was used to incorporate the effect of variation of outcome by different ICUs.

Results:

	Calibration and discrimination statistics			
Models	HL statistics	*P*-value for HL	c-index (ROC)	95% CI of c-index (ROC)
MLR	6.14	0.63	0.845	0.821–0.868
Bootstrapped LR	11.99	0.15	0.840	0.816–0.863
UK APACHE II	256.54	<0.000	0.813	0.789–0.838

The variation of outcome by ICU was not statistically significant ($P = 0.28$). Using a 10 per cent mortality cut-point for ICU admission, we anticipate 50 per cent reduction in ICU admissions with an observed HDU mortality of 6 per cent.

Discussion: The models developed can reliably risk-stratify postoperatively both emergency and elective AAA repairs. This has enormous impact for the vascular service by: (1) formally defining low-risk cases for HDU management, and (2) offering the means to design and analyse a definitive randomized trial of low-risk cases between HDU and ICU care.

Vascular 16

Natural history of nonoperated abdominal aortic aneurysm

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Aim: To assess the outcome for patients with nonoperated asymptomatic and symptomatic abdominal aortic aneurysm (AAA).

Methods: Patients admitted between January 1995 and December 1999 with an AAA, who did not have surgical repair, were included. Demographic data, aneurysm size, reason for no operation, follow-up duration and cause of death were examined. Survival data were obtained from general practice and hospital records. Cause of death was identified from the General Register Office for Scotland.

Results: Of the 758 patients admitted with AAA, 128 had intact lesions and did not undergo operation. These comprised 77 men and 51 women of median (range) age 73 (60–92) years; 75 (59 per cent) had an asymptomatic and 53 (41 per cent) had a symptomatic lesion. The median (range) AAA diameter was 55 (27–85) mm for asymptomatic and 55 (33–100) mm for symptomatic lesions. The commonest reasons for not operating were significant comorbidity in 73 (57 per cent) and small aneurysm size in 32 (25 per cent). Overall, follow-up was for a median (range) of 19 (1–74) months. In the asymptomatic group, 31 (41 per cent) patients were alive, 26 (35 per cent) having died from a cause unrelated to the AAA and 15 (20 per cent) from the AAA; 3 (4 per cent) were lost to follow-up. In the symptomatic group, 18 (34 per cent) were alive, 23 (43 per cent) having died from an unrelated cause and 12 (23 per cent) from the AAA. Of the 127 patients in both groups, only 27 (21 per cent) died from their AAA.

Conclusion: Only one-fifth of patients with AAA who were not operated had an aneurysm-related death.

Vascular 17

Prospective randomized trial of superficial femoral artery (SFA) angioplasty with adjuvant photodynamic therapy (PDT) using 5-aminolaevulinic acid (ALA): safety and tolerability

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Aims: The clinical feasibility of PDT as an adjunct to SFA angioplasty has been demonstrated in a small pilot study. We are now undertaking a randomized controlled trial of superficial femoral artery angioplasty with adjuvant PDT.

Methods: One hundred and thirty patients with symptomatic SFA stenosis or a short (<4 cm) occlusion will be randomized to receive the photosensitizer 5-ALA or placebo doses every 3 h commencing 5 h prior to angioplasty. In the patients receiving active drug, angioplasty is immediately followed by intraluminal light delivery (50 J cm^{-2} at 635 nm). The control group undergoes a standard angioplasty only. Lesions are assessed by Duplex ultrasound scanning, before the procedure and at 24 h, 1, 3 and 6 months afterwards. An interim procedural safety review was carried out of 30 patients.

Results:

	Treatment group	Control group
Number (males)	15 (9)	15 (6)
Median age (range) in years	74 (55–80)	76 (55–89)
Risk factors		
Known IHD	9	5
Treated hypertension	12	8
Known diabetes	3	4

The illumination was tolerated well, although three patients complained of local pain in the treatment group. There were no vascular complications. ALA-induced hypotension was observed in six patients who received treatment. The hypotension responded promptly to IV fluid. Diuretics and antihypertensives potentiate the hypotension and may be best omitted on the day of treatment.

Conclusion: SFA angioplasty with adjuvant PDT is safe and well tolerated. It is clinically feasible and only increases treatment time by 10–15 min.

Vascular 18

Angioplasty of iliac occlusions: a case for surgery

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Aims: Primary angioplasty (PTA), stenting and surgical revascularization have all been advocated for the treatment of iliac occlusions. The aim of the study was to review the long-term outcome of a policy of primary angioplasty with salvage stenting as the first-line treatment for iliac occlusions.

Methods: Between 1996 and 2000, 90 patients underwent PTA for iliac occlusions in 94 limbs (median age 65 (43–88) years). Presenting symptoms were claudication in 79 and critical limb ischaemia in 15. There were 16 diabetics. Short-term complications and long-term outcome for the patient cohort were defined by clinical follow-up. Results were quantified by life table analysis, and groups were compared by the Kaplan–Meier test.

Results: Technical success was obtained in 77 per cent of the patient group. Salvage stent placement was utilized in 17 per cent of the cases. There were five immediate complications that required surgical intervention (5 per cent) and one death within 30 days (1 per cent). Cumulative limb salvage, patient survival and symptom recurrence rates at 1 and 4 years were 99, 89 and 39, and 99, 64 and 41 per cent, respectively. There was a significant difference in symptom recurrence between those patients having PTA of the CIA when compared to the EIA (12-month symptom recurrence rates, 27 per cent CIA *versus* 47 per cent EIA, $P = 0.025$). Mode of presentation, run off and presence of diabetes did not affect symptom recurrence ($P > 0.05$).

Conclusion: Recurrent symptoms are common following PTA of external iliac occlusions. These lesions may be better treated with surgical revascularization.

Vascular 19

Venous claudication in iliofemoral thrombosis: long-term effects on venous haemodynamics, clinical status and quality of life

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Aims: We evaluated long-term impact of iliofemoral thrombosis (IF_{DVT}) on walking capacity, venous haemodynamics, CEAP class, venous-clinical-severity score (VCSS) and quality of life (SF-36), and determined prevalence of venous claudication.

Methods: All patients with prior IF_{DVT} at our institution since 1990 were followed up. Excluded were those with walking impairment due to arterial (ABI < 1.0 post-exercise) or unrelated causes, and those thrombectomized or thrombolyzed. Thirty-nine patients (22–83 years) were included. Median follow-up was 5 years (range: 1–23). Investigation included CEAP and VCSS stratification, air-plethysmography (outflow fraction: OF; venous-filling index: VFI; residual-volume fraction: RVF), duplex, treadmill (3.5 km h^{-1}, 10 per cent) to determine initial (ICD) and absolute (ACD) claudication distances, and SF-36 assessment. Non-affected limbs of patients with unilateral IF_{DVT} (37/39) comprised the control group; data presented as median and interquartile range.

Results: A total of 75.6 per cent of limbs with IF_{DVT} had superficial and deep reflux and 26.3 per cent superficial reflux; reflux in control limbs was 13.5 and 19 per cent, respectively ($P < 0.01$); 43.6 per cent (17/39) (95 per cent CI: 27–60 per cent) of patients developed venous claudication ipsilateral to IF_{DVT} (ICD: 130 (105–268) m), compelling 15.4 per cent (6/39) (95 per cent CI: 3.5–27 per cent) to discontinue treadmill (ACD: 241 (137–298) m). Limbs with prior IF_{DVT} had a lower OF (37 (32.2–43) per cent; $P < 0.001$), abnormally higher VFI (3.8 (2.5–5.7) mL s^{-1}; $P < 0.001$) and RVF (45 (32.5–51.5) per cent; $P = 0.006$), and clinical impairment in CEAP and VCSS ($P < 0.0001$). Patients with IF_{DVT} had impaired physical functioning and role ($P < 0.034$), general health ($P < 0.001$), social function ($P = 0.047$) and mental health ($P = 0.043$).

Conclusions: Of those with prior IF_{DVT} 43.6 per cent developed venous claudication, compelling interruption of walking in 15.4 per cent; prior IF_{DVT} caused outflow impairment, and large residual-venous volume and reflux, resulting in marked clinical and quality-of-life compromise. Standardized challenge enabled discrimination of those with clinically relevant impairment.

SARS: Miscellaneous 01–04

Wednesday, 22 May 17.05–18.15

Miscellaneous 01

Local or general anaesthetic for open hernia repair – a randomized trial

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Background: Local anaesthetic hernia repair is thought to be safer for patients, causes less postoperative pain, costs less and is associated with a more rapid recovery for patients when compared with the same operation performed under general anaesthetic. The aim of this study was to compare patient outcome following local (LA) or general anaesthetic (GA) repair of a primary groin hernia.

Methods: A randomized trial comparing patient outcome following LA or GA hernia repair. Outcome parameters measured included tests of vigilance, divided attention, sustained attention, memory, cognitive function, pain, return to normal activity and costs.

Results: Two hundred and seventy-nine patients were randomized to LA or GA hernia repair, 276 of whom had an operation with 138 participants in each group. At 6, 24 and 72 h postoperatively there were no differences in vigilance or divided attention between the groups. Similarly, memory-sustained attention or cognitive function was not impaired in either group. Although physical activity was significantly ($P = 0.0001$) impaired at 24 h, this and return to usual social activities, 10 (7–14) days for LA *versus* 11 (7–14.5) days for GA, $P = 0.5841$, were similar in both groups. While patients in the LA group had significantly less pain on moving ($P = 0.04$) at 6 h, they were less likely 84 per cent *versus* 95 per cent for GA ($P = 0.011$), to recommend the same operation to someone else. General anaesthetic hernia repair cost £30.54 more than the same operation under LA.

Conclusions: This study indicates that patient recovery is similar after LA or GA hernia repair.

Miscellaneous 02

Re-epithelialization of cutaneous wounds is augmented by hypoxia

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Background: Human keratinocytes have been shown to demonstrate increased motility in hypoxic wounds correlating with the success of semiocclusive hypoxic dressings, although the underlying mechanism remains unknown. The authors propose that the av subgroup of integrins, suggested to have an inherent modulatory function in migration, as well as a regulatory role in matrix metalloprotease (MMP) activation, is a key step in re-epithelialization of hypoxic wounds.

Methodology: Sub-confluent human keratinocyte cell monolayers were exposed to hypoxia for 24 h. Reoxygenation studies were performed up to 72 h. Control cells were left in normal growth conditions. Cellular av subunit and avβ6 expression was measured by flow cytometry. Migration scratch assays on fibronectin (avβ6 substrate) posthypoxic exposure were performed over 24 h. Relative MMP-2, 9 secretion were estimated using gelatin zymography. Inhibition studies were performed using a specific blocker to avβ6 (MAB10D5). ANOVA and Mann–Whitney *U*-tests were used as indicated.

Results: Cell viability was not affected up to 72 h posthypoxic insult. Sustained increases in av and avβ6 were demonstrated up to 48 h in reoxygenation studies ($P < 0.001$). Standardized scratch assay confirmed increased migration in the hypoxic group ($P < 0.05$). This effect was attenuated by the addition of a specific inhibitor to avβ6. MMP-2, 9 secretion was up-regulated posthypoxic exposure ($P < 0.001$, $P < 0.05$). Increased MMP expression was attenuated by addition of an avβ6 inhibitor ($P < 0.05$).

Conclusions: These results indicate that avβ6 and MMP-2,9 up-regulation are integral features of increased migration in hypoxic human keratinocytes thus identifying potential therapeutic targets for those with nonhealing wounds.

Miscellaneous 03

A morphological study of hyperhidrotic and normal human apocine sweat glands

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Background: Studies in hyperhidrosis have demonstrated that eccrine glands express all the signs of chronically activated normal glands but that the glands are otherwise morphologically normal. This study is the first to compare the morphology of normal and hyperhidrotic apocrine sweat glands.

Methods: Apocrine sweat glands were isolated from the axilla of normal patients and hyperhidrotic individuals undergoing thoracic sypathectomy. Glands were wax embedded, sectioned (5 μm) and digital images analysed using analysis digital imaging software. All data are represented as mean ± s.e. from ≥30 observations. All tests of significance are Students *t*-test.

Results: Hyperhidrotic glands were 30 per cent larger than normal in both length and width (length – normal 906 ± 71 μm *versus* 1173 ± 88 μm, $P < 0.03$ width – normal 613 ± 55 μm *versus* 811 ± 49 μm, $P < 0.02$). The tubule of the hyperhidrotic glands was significantly thicker (106.2 ± 2.6 μm *versus* 93.5 ± 4.9 μm; $P < 0.03$), whereas the hyperhidrotic lumen was 40 per cent wider than normal (56.6 ± 2.7 μm *versus* 40.6 ± 4.4 μm, $P < 0.002$). Excessive collagen matrix was excluded by measuring tubule area which was 60 per cent larger for hyperhidrotic glands compared to normal (257 575 ± 18 338 μm^2 *versus* 162 304 ± 12 971 μm^2, $P < 0.01$) indicating that the hyperhidrotic glands were more tightly wound than normal.

Conclusions: Apocrine sweat glands are not anatomically different in hyperhidrosis but are significantly larger, more coiled and have a larger luminal diameter. The cells also appear degranulated and more squamous suggesting increased activity. Hyperhidrosis is associated not only with increased eccrine activity but also affects apocine function.

Miscellaneous 04

Laparotomy and laparoscopy differentially affect perioperative tumour angiogenesis

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Background: Surgical trauma potentiates tumour growth. Endothelial progenitor cells (EPC), derived from bone-marrow, enhance tumour angiogenesis and growth. We hypothesized that laparotomy and laparoscopy differentially affect the mobilization of EPCs and hence tumour angiogenesis.

Methods: C57BL/6 mice, bearing Lewis lung (3LL) flank tumours, were randomized to laparotomy, laparoscopy (CO_2 pneumoperitoneum), and anaesthetic-only treatment groups ($n = 12$ per group). Bone marrow EPC were detected by flow cytometry using stem cell antigen (SCA-1) at 6, 24 and 48 h after treatment. Differentiated EPC were identified, at the same time points, in splenic homogenates by dual staining for lectin/UEA-1 and acetylated low density lipoprotein uptake. These findings were correlated with changes in tumour volume and microvessel density for each group. In addition, EPC uptake in tumours was evaluated using CM-DiI-labelling. This was further correlated with tumour growth and angiogenesis.

Results: Laparotomy induced an 11-fold ($P = <0.001$) increase in bone marrow EPC, compared with anaesthetic-only control (table) at 24 h. Laparoscopy significantly ($P = <0.001$) attenuated EPC mobilization compared with laparotomy. This correlated with reduced tumour growth and angiogenesis. Following laparotomy, increased EPC were demonstrated lining tumour microvessels. This phenomenon was significantly ($P = <0.001$) attenuated in the laparoscopy group.

	EPC positivity (% of control)		
	6 h	24 h	48 h
Laparotomy	234+/12.3*	1123+/21*	162 ± 8.6*
Laparoscopy	110+/4.6*	454 ± 9 1*	150 ± 6.8*
Control	100 ± 0	100 ± 0	100 ± 0

Data; mean ± standard error of mean Statistics one-way ANOVA *$P < 0.05$ versus control.

Conclusions: Laparotomy is associated with significant mobilization of EPC for perioperative tumour angiogenesis and growth. Laparoscopy attenuates this effect These findings identify a novel pathway by which open surgery promotes tumour angiogenesis.

SARS: Vascular 01–04

Vascular 01

Inflammatory response following lower limb ischaemia-reperfusion injury: role of intestinal mucosal barrier

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Aim: To investigate intestinal mucosal barrier function following isolated lower limb ischaemia-reperfusion (I/R) injury

Materials and methods: Intestinal permeability was assessed by lactulose/mannitol test pre- and postoperatively in three groups of patients group I, patients with critical limb ischaemia (CLI) undergoing infra-inguinal bypass surgery ($n = 18$), group II, patients with intermittent claudication undergoing infra-inguinal bypass surgery ($n = 14$); and group III control, patients with symptomatic carotid stenosis undergoing carotid endarterectomy ($n = 13$) Systemic endotoxins were meaured at PO and daily until day 7. Signs of systemic inflammatory response syndrome (SIRS) were also recorded daily This study was approved by the local ethical committee

Results: A significant increase in intestinal permeability was observed in group I at day 3 (0 04(0 03)) compared to PO (0 01(0.05)), Wilcoxon signed ranks test) This was also found to be significant when compared to group II (0.02(0 01), $P < 0.05$, Mann–Whitney *U*-test), which showed no significant change at any of the time points Systemic endotoxaemia was not significantly detected in any of the groups Postoperatively, 39 per cent of patients developed SIRS in group I, compared to 14 and 8 per cent in group II and III, respectively ($P < 0.05$, Chi-square test).

Conclusions: The results confirm that lower limb I/R injury is associated with an increase in intestinal permeability in CLI only They suggest that the gut may play a role in the development of SIRS in these patients They also suggest that the magnitude of I/R injury is related to the degree of initial ischaemia

Vascular 02

Taurine attenuates intestinal hyperpermeability induced by ischaemia/reperfusion

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Background: Ischemia/reperfusion (I/R) mediated gut damage is an unevitable consequence of resuscitation and certain form of aortic reconstructive surgery The resultant increase in gut permeability has been implicated in systemic inflammatory response syndrome and multiple organ failure Taurine modulates the pro-inflammatory effects of I/R and also is protector of stressed enterocytes We examined its effects in an experimental model of I/R-induced injury.

Methods: Sprague–Dawley rats were randomized into three groups Control; I/R, Taurine plus I/R Taurine (200 mg kg^{-1} per day) was given for 5 days before I/R I/R was induced by cross-clamping superior mesenteric and coeliac vascular pedicle for 30 min, followed by 3-h reperfusion The magnitude of intestinal permeability of a segment of jejunum was determined by measurement of the ratio of plasma to lumen expression of the fluorescent dextran (FD4) using luminescence spectrophotometer A laser-Doppler monitor measured the intestinal blood flow, expressed as flux Intestinal oedema was determined by wet . dry ratio of the gut tissue.

Results: At the end of reperfusion:

	Control	I/R	Taurine + I/R
IP (%)	6 00 ± 1 23*	37 60 ± 7 68	11 40 ± 2 29*
Flux (%)	78 88 ± 3 40*	43 24 ± 2 47	69 20 ± 4 16**
Wet dry ratio	3 69 ± 0 15	5 26 ± 0 54	3 65 ± 0 29*

Data are mean ± SEM Statistics ANOVA with Scheffé post hoc test
$^{*}P < 0\ 01$ and $^{**}P < 0\ 05$ *versus* I/R

I/R significantly decreased gut blood flow, increased the intestinal permeability, and oedema Administration of taurine significantly attenuated these events

Conclusion: These data demonstrated that taurine protected against I/R-induced decrease in gut blood flow and increase in intestinal permeability and the mechanism underlying this protection warrants further investigation

Vascular 03

The role of matrix metalloproteinases in the natural history of abdominal aneurysms

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Matrix metalloproteinases (MMPs) play an important part in the expansion of abdominal aortic aneurysms (AAA) It has been suggested that MMPs appear to be present in differing concentrations during the progression of aneurysm development Our aim was to determine the MMP profiles in tissue from aneurysms of differing sizes, to define whether rupture results from gradual widespread increases in proteolytic capacity, or is confined to a localized area of the aortic wall Samples of anterior aortic wall were obtained from 81 patients undergoing elective AAA repair ($n = 59$, median age 72 years, diameter 6 cm), or emergency repair of ruptured AAA (rAAA) ($n = 22$, age 72) The elective group was divided by aneurysm size into 2 groups, medium sized (5–6 5 cm, $n = 31$, age 70) and large (>6.5 cm, $n = 11$, age 73). Paired samples of aortic sac were obtained from the sac and the site of aortic rupture from nine patients with rAAA (age 70). MMPs-1, -2, -3, -9, and -13, and TIMPs-1 and -2 were extracted from the tissue specimens and quantified using ELISA. There were no significant differences in MMP and TIMP levels in AAA sac of medium and large sized aneurysms, or ruptured and nonruptured AAA sac In paired specimens taken from rAAA, MMP-9 was seven times higher at the site of rupture than in the anterior sac (98.57 ng mg^{-1} [IQR 56 02–134 5] *versus* 13 89 ng mg^{-1} [7 80–46.81], $P = 0\ 012$). These data demonstrate that MMP profiles vary little with expansion of the aneurysm in medium- and large-sized aneurysms Rupture is likely to be a result of localized elevations in MMP-9 and this would be an appropriate target for pharmacotherapy

Vascular 04

Biochemically observed renal protective effects of methylprednisolone are not detectable by early histopathology, in a porcine model of infrarenal aortic ischaemia-reperfusion

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Background: Methylprednisolone (MP) reduces subclinical biochemical renal injury as measured by urinary *N*-acetyl-beta-D-glucosaminidase/creatinine ratio (NAG/creatinine) at porcine vascular surgery. Tubular dilatation is a recognized marker of the severity of acute tubular injury. We hypothesized that MP might, in addition to reducing biochemical injury, reduce histological renal injury in a porcine model of infrarenal aortic ischaemia-reperfusion

Methods: Forty-two pigs underwent anaesthesia, laparotomy, normodynamic fluid resuscitation for 60 min and then 150 min of infrarenal aortic cross-clamping followed by 180 min of reperfusion Pigs were randomized to two groups Group C received placebo and group MP received methylprednisolone 30 mg kg^{-1} Urine was collected for measurement of NAG/creatinine and the left kidney excised, for histological examination, after 180 minutes of reperfusion The ratio of total luminal to tubular area (as a marker of tubular injury) was calculated for each slide using image analysis software (Adobe Photoshop 5 0) Analysis between groups was with Mann–Whitney *U* Data were expressed in median [interquartile range].

Results: There were no haemodynamic differences between groups Total urinary output was significantly lower in group C as compared to group MP Consistent with our previous report, NAG/creatinine was lower in the MP group. However, there was no significant difference in renal injury ratios between group C (0.237 [0.216–0 265]) as compared to group MP (0 248 [0.229–0 270]) There was no correlation between NAG/creatinine and histological findings

Conclusion: Urinary NAG/creatinine ratios may be a more sensitive indicator of possible renal protective effects of MP than early histopathology at vascular surgery.

SARS: Miscellaneous 05–08

Wednesday, 22 May 17.05–18.15

Miscellaneous 05

The effect of cytokine gene polymorphisms on the inflammatory response to elective abdominal aortic aneurysm repair

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Background: Many cytokine genes contain polymorphic sites, some of which have been shown to affect transcription *in vitro*. The aim of this study was to determine whether cytokine polymorphisms affect circulating levels of cytokines *in vivo* after abdominal aortic aneurysm (AAA) repair.

Methods: Seventy-four patients undergoing elective AAA repair had blood samples taken at induction of anaesthesia and 24 h after surgery. Plasma interleukin-6 and tumour necrosis factor-alpha (TNF-α) levels were determined by ELISA. Genotypes for the single-nucleotide polymorphisms at the following loci were determined using induced heteroduplex genotyping: IL-6-174 and TNF-α-308. Patient cytokine genotypes were compared with their baseline phenotypes and the change in cytokine levels between induction and 24 h postoperatively. Cytokine levels are expressed as mean (SEM) or median (range).

Results: Patients with a IL-6-174 'G' allele (56 patients, 77 per cent) had higher mean baseline plasma levels of IL-6: 16.06 pg/mL (9.7) *versus* 0.849 pg/mL (0.85), $P = 0.124$; and the mean change in plasma IL-6 between induction and 24 h was also higher for these patients: 370.55 pg/mL (46.87) *versus* 290.44 pg/mL (37.11), $P = 0.185$. Those patients with a TNF-α-308 'A' allele (26 patients, 35 per cent) had higher median baseline plasma levels of TNF-α: 2.49 pg/mL (0–163.5) *versus* 0.0 pg/mL (0–159.57), $P = 0.196$, and the mean change in plasma TNF-α between induction and 24 h was also higher for these patients: 24.67 pg/mL (12.67) *versus* 17.06 pg/mL (7.09), $P = 0.60$.

Conclusion: Cytokine genotype may affect *in vitro* cytokine production in the resting physiological state and in response to surgical stress.

Miscellaneous 06

Delayed graft function after cadaveric kidney transplantation

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Background: Delayed graft function (DGF) commonly occurs after renal transplantation and it has been associated with reduced short- and long-term graft survival. The aim of this study was to identify the incidence, causes and effects of DGF in cadaveric renal transplants within the renal transplantation unit of the Royal Infirmary of Edinburgh.

Methods: A study group of 181 renal transplant recipients transplanted from 173 cadaveric donors, in the period between January 1994 and December 1999, was analysed. Several donor and recipient factors were selected and analysed with appropriate statistical tests. A P-value of <0.05 was considered statistically significant.

Results: The incidence of DGF was 41 per cent. Factors significantly related to the occurrence of DGF include donor age greater than 40 years old ($P = 0.041$), female donor ($P = 0.025$), donor with comorbidities ($P = 0.029$), higher donor-recipient HLA-mismatch grade ($P = 0.007$), longer recipient duration on dialysis ($P = 0.001$) and recipient who had been transplanted before ($P = 0.004$). Graft survival in patients with immediate graft function at 3 months, 6 months and 1 year after transplantation was 94, 94 and 91 per cent, respectively. The graft survival in patient with DGF was approximately 12 per cent lower for each survival time. In addition, the patient's 1-year survival rate was 97 per cent for the immediate function group, and 96 per cent for the DGF group.

Conclusions: DGF has a significant impact on the graft survival, as the majority of donor and recipient factors significantly associated with DGF are unavoidable. Machine perfusion may play a role in DGF reduction and warrants further studies.

Miscellaneous 07

Ischaemia–reperfusion injury, gut macromolecular permeability and severe acute pancreatitis

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Background: Splanchnic ischaemia–reperfusion injury (I-R) is postulated as a potential cause of altered gut permeability and the subsequent translocation of bacteria, associated with a severe attack of acute pancreatitis (AP). The current study aims to assess the relationship between gut macromolecular permeability, urine intestinal fatty acid binding protein concentration (IFABP-c, a novel marker of I-R mediated gut mucosal injury), and severity of attack of AP.

Methods: Patients with AP were studied within 72 h of pain. Polyethylene glycol (PEG) probes (3350 and 400 kDa) were administered enterally, and the percentage retrieval ratio after renal excretion was used as a measure of intestinal macromolecular permeability. The collected urine was also used to determine IFABP concentration and total IFABP excreted. The magnitude of the systemic inflammatory response was estimated by peak 0–72 h CRP, and severity of attack was classified as mild or severe (Atlanta criteria).

Results: Sixty-one patients with AP (19 severe) and 12 healthy controls were studied. Compared with mild attacks, a severe attack was associated with significantly higher PEG excretion ratios ($P < 0.001$), PEG 3350 per cent-retrieval ($P < 0.001$), urine IFABP-c (median: 85 ng mL^{-1} *versus* 1086 ng mL^{-1}, $P < 0.001$), and total IFABP (median: 210 mg *versus* 1143 mg, $P = 0.002$). Healthy controls had lower urine IFABP-c and total IFABP compared with mild AP ($P < 0.01$). Furthermore, urine IFABP-c correlated significantly with PEG 3350 per cent-retrieval ($r = 0.50$, $P < 0.001$), PEG retrieval ratio ($r = 0.3$, $P = 0.04$), and peak CRP ($r = 0.51$, $P < 0.001$).

Conclusion: It appears that splanchnic hypoperfusion and ischaemia–reperfusion injury contribute to the observed increase in intestinal permeability associated with severe AP.

Miscellaneous 08

Heat shock protein-60 peptide induces interleukin-10 production in inflammatory bowel disease: evidence of a self-antigen-specific regulatory response

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Background: It has been known for some time that mucosal T-cells specific to mycobacterial heat shock proteins (hsp) are induced in inflammatory bowel disease (IBD). Hsp-60 peptide therapy has been shown to induce a T helper 2 cytokine profile characteristic of remission in autoimmune diseases. As human hsp60 is highly homologous with mycobacterial hsp60 and is abundantly expressed following tissue injury, we hypothesized that self-hsp60 may play a role in regulating acute inflammation in IBD.

Methods: Peripheral blood mononuclear cells (PBMC) were harvested with informed consent from 11 patients undergoing surgery for acute IBD and compared to normal controls. Cells were cultured for 5 days with recombinant human hsp60 antigen and proliferation was measured by 3H-thymidine incorporation. Cultures were restimulated for a further 48 h to amplify a hsp60-specific T-cell population, and cytokine production was measured by immunoassay. In addition, mesenteric lymph node cells were harvested from five patients and hsp60-specific cytokine production was assessed after 3, 5 or 7 days of culture.

Results: Control PBMC proliferated well in response to hsp60 (142 per cent of unstimulated) while IBD PBMC proliferated poorly (99 per cent of unstimulated). Production of the anti-inflammatory cytokine IL-10, but not TGF-β, was increased (3.1(2) ng/mL *versus* 2.6(1) control) while pro-inflammatory IFN-γ production was decreased (9.4(2.5) ng mL^{-1} *versus* 17.9(6.6) control). Mesenteric lymph node IL-10 production was increased at all time points compared to IFN-γ production.

Conclusion: Our data provides evidence of a self-antigen-specific immune response which may act *in vivo* as an anti-inflammatory regulatory mechanism in IBD.

ASGBI: Six of the Best AUGIS, Upper GI 13–18

Thursday, 23 May 08.30–10.15

Six of the Best, Upper GI 13

Diclofenac reduces the incidence of post-ERCP acute pancreatitis

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Aim: To determine whether diclofenac can reduce the incidence or severity of post-ERCP acute pancreatitis.

Methods: A prospective, blind, randomized, controlled trial comparing diclofenac with a placebo was conducted. To maximize the incidence of pancreatitis, entry into the trial was restricted to patients who underwent retrograde pancreatography or with manometrically verified sphincter of Oddi hypertension. After endoscopy, a suppository containing either 100 mg of diclofenac or a placebo was administered. Serum amylase and clinical parameters were checked at 2 and 24 h. The internationally accepted definition of acute pancreatitis was observed.

Conclusion: Two hundred patients entered the trial, 100 receiving rectal diclofenac. The two groups were well matched for factors which might increase the risk of post-ERP acute pancreatitis. Twenty-three patients developed acute pancreatitis (11.5 per cent), of whom seven (3.5 per cent) received rectal diclofenac and 16 (8 per cent) a placebo ($P < 0.05$). Six patients with sphincter of Oddi hypertension developed acute pancreatitis, three in each group. Excluding sphincter of Oddi hypertension patients, the incidence of pancreatitis falls to 2 per cent in the diclofenac group and 6.5 per cent in the placebo group ($P < 0.03$). Two of 23 patients developed severe pancreatitis. Both of these patients were in the control group. There was no mortality and all patients were discharged home. The average inpatient stay for patients who developed acute pancreatitis was 3 days for the diclofenac group and 5 days for the control group.

Conclusion: This trial suggests that rectal diclofenac given immediately after ERCP reduces the incidence, hospital stay and severity of pancreatitis in higher risk patients without sphincter of Oddi hypertension.

Six of the Best, Upper GI 14

Influence of the type of surgery and surgical complications in the ESPAC-1 adjuvant trial

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Aims: The ESPAC-1-randomized controlled trial of 541 patients with pancreatic ductal adenocarcinoma showed that adjuvant chemotherapy increased median survival compared to no chemotherapy (19.7 months *versus* 14.0 months, $P = 0.0005$), including those with R1 resection margins but chemoradiotherapy did not. The aim of this study was to determine if postoperative complications or the type of surgery affected long-term survival.

Methods: Analyses of patients on the ESPAC-1 database by categorical, log-rank and Cox proportional hazards modelling.

Results: Operative details were available on 466 (86 per cent) patients. Kausch–Whipple (KW) was performed in 241 (52 per cent of total), pylorus-preserving KW (PPKW) in 171 (37 per cent), distal pancreatectomy in 35 (7 per cent) and total pancreatectomy in 19 (4 per cent) patients. Postoperative complications occurred in 125/446 (28 per cent) patients including. fistula = 37, delayed gastric emptying = 24, pulmonary complications = 14, and bleeding = 11. Survival analysis showed no significant difference in survival (median, 95 per cent CI) between those that had complications (17.2, 14.0–22.2 months) and those that did not (15.7, 13.7–17.4 months; $P = 0.073$). Patients that had a PPKW resection had a longer survival (18.1, 16.5–21.7 months) than those that had a KW resection (14.1, 12.8–16.4 months, $P < 0.001$). Stepwise selection revealed that only three factors were of independent prognostic significance: tumour grade ($P < 0.001$), nodal status ($P < 0.001$) and tumour size ($P = 0.013$) but not PPKW ($P = 0.07$).

Conclusions: Patients who had complications after resection for pancreatic cancer still benefited from adjuvant treatment. Moreover, there was no evidence from this trial that the more conservative PPKW resulted in reduced long-term survival.

Six of the Best, Upper GI 15

Early changes in intestinal permeability to macromolecules predict outcome in patients with acute pancreatitis: a comparative study with C-reactive protein and the APACHE II scoring system

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Aims: The aim of this study was to investigate whether these early changes in intestinal permeability predict outcome of the attack, and to compare that with C-reactive protein (CRP) and the APACHE II scoring system.

Methods: Intestinal permeability to macromolecules (polyethylene glycol 3350) was determined within 72 h of onset of symptoms in 85 patients with acute pancreatitis. Serum CRP was measured daily, and a peak within 120 h was identified. APACHE II scores were determined at 24 h. Attacks were classified as mild ($n = 57$) or severe ($n = 21$) according to the Atlanta criteria.

Results: Patients with severe attacks had significantly greater peak serum CRP levels (median 260 mg L^{-1} *versus* 93 mg L^{-1}, $P < 0.0001$), intestinal permeability index (0.06 *versus* 0.008; $P < 0.0001$), and APACHE II scores (10 *versus* 5; $P < 0.001$) compared to mild attacks. The areas under the ROC curve for the prediction of severe attacks, the development of sepsis, organ failure or death, and a prolonged hospital stay were comparable between the three prognostic variables.

Conclusions: Early changes in intestinal permeability to macromolecules in patients with acute pancreatitis are highly predictive of severity and outcome of the attacks, and have a predictive power that is comparable to that of the APACHE II scoring system and serum CRP. Early therapeutic interventions aimed at restoration of the gut barrier function might have a favourable impact on the course of the disease.

Six of the Best, Upper GI 16

Chemotherapy and radiotherapy followed by surgical excision (CARE): a phase II trial in the treatment of oesophageal carcinoma

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Introduction: The benefits of neoadjuvant therapy in the treatment of patients with resectable oesophageal carcinoma are controversial. Patients with locally advanced carcinomas were entered into a phase II trial of preoperative chemotherapy and radiotherapy followed by oesophagectomy.

Methods: Fifty-five patients aged <70 years with T3 ± N1 oesophageal tumours were recruited between February 1998 and July 2001. Patients received 12 weeks of chemotherapy with four cycles of cisplatin 60 mg m^{-2} plus infusional 5FU (300 mg m^{-2}, days 1–42; 225 mg m^{-2}, days 43–75). Radiotherapy 45Gy was administered on days 43–75. Two stage en-bloc oesophagectomy with two field lymphadenectomy was undertaken 6 weeks after neoadjuvant therapy.

Results: Forty-one patients (74 per cent) completed the treatment. Two patients (3.6 per cent) died during neoadjuvant therapy (SVC obstruction, perforated diverticular disease), while four withdrew due to toxicity. In eight patients (14.5 per cent), the tumour progressed on therapy. Overall mortality was 5/55 (9.1 per cent) with a postoperative mortality of 3/41 (7.3 per cent). Significant postoperative morbidity was observed in 19/41 (46.3 per cent). The tumour was downstaged in 34/41 (83 per cent) with a pathological complete response in 26.8 per cent. Median lymph node yield was 18, there was no proximal or distal margin involvement and a positive circumferential margin in 25 per cent. Median survival on an intention to treat basis was 26 months, and it was 38 months in the patients who underwent surgical resection.

Conclusions: Preoperative neoadjuvant therapy followed by en-bloc oesophagectomy can be undertaken with acceptable levels of morbidity and mortality. The survival data appears promising and this study should act as a platform for the initiation of a phase III multicentre trial.

Six of the Best, Upper GI 17

Initial results of a randomized trial of laparoscopic total (NISSEN) *versus* posterior partial (TOUPET) fundoplication for gastro-oesophageal reflux disease

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Aims: To prospectively compare a total and a posterior partial laparoscopic fundoplication in the control of symptomatic gastro-oesophageal reflux disease.

Patients and methods: A total of 127 patients randomized to laparoscopic 360° (Nissen) ($n = 64$) or 270° (Toupet) ($n = 63$) fundoplication. Symptomatic assessment at 6 weeks, 6 months and 1 year. Postoperative 24-h pH studies performed at 6 months.

Results: No significant differences were seen in heartburn, regurgitation, dysphagia, epigastric pain, postprandial fullness, gas-bloat, inability to burp, increased flatus, diarrhoea or abdominal pain at 6 weeks, 6 months and 1 year postoperatively. Chest pain on eating was more common at 1 year in the 360° group ($P = 0.03$). There were seven failures on postoperative pH criteria, two in the 360 group and five in the 270 group.

Conclusions: Any differences in the symptomatic outcome of laparoscopic total and posterior partial fundoplication are minimal. A 360° fundoplication is less likely to fail to control gastro-oesophageal reflux on postoperative pH criteria.

Six of the Best, Upper GI 18

Laparoscopic adrenalectomy: a prospective evaluation of 151 procedures

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Aims: To report our experience with laparoscopic transperitoneal adrenalectomy.

Methods: From November 1993 to August 2000, 151 laparoscopic adrenalectomies were attempted in 132 patients who presented with symptomatic adrenal masses or who had an incidental large mass diagnosed during investigations for other complaints. All perioperative and follow-up data was prospectively recorded on a dedicated unit database.

Results: The median age was 52 years (18–77 years). Seventy-six per cent were female. Lesions were left-sided in 48 per cent of patients and bilateral in 14 per cent. Indications for resection were: Conn's syndrome (54), phaeochromocytoma (27), Cushings disease (13), nonfunctioning adenoma (14), congenital adrenal hyperplasia (2), cortisol-producing adenoma (5), combined Cushings and Conn's syndrome (1), primary or metastatic carcinoma (7), benign cyst/lipoma (8), and no lesion (1). Median size of the lesions was 3.0 cm (0.5–20 cm). Median operating time was 65 min (30–170 min). Conversion to an open procedure was necessary in 10 patients (8 per cent). Minor morbidity occurred in nine patients (7 per cent). Major morbidity occurred in two patients (pancreatitis, peritonitis). Median hospital stay was 3 days (1–16 days). At median follow up of 28 months (1–94 months), five patients (4 per cent) had persistent hypertension. No patient had evidence of recurrent hormonal excess.

Conclusions: Laparoscopic removal of the adrenal gland should be considered the surgical procedure of choice in experienced centres. It requires a high degree of technical expertise and should remain within the remit of the advanced laparoscopic surgeon.

ASGBI: Moynihan 01–08

Thursday, 23 May 08.30–10.30

Moynihan 01

The sentinel node concept in the metastatic dissemination of oesophageal adenocarcinoma

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Aims: The sentinel node concept states that the first lymph node on a direct pathway from a tumour will be the first site of lymphatic spread. The aim of this study was to determine the feasibility and accuracy of sentinel node biopsy for oesophageal adenocarcinoma.

Methods: Fifty-seven patients (median age 62 years; M : F = 4.1) with adenocarcinoma of the lower oesophagus ($n = 40$) or cardia ($n = 17$) underwent endoscopic peritumoural injection of ^{99m}Tc-nanocolloid prior to en-bloc resection with extended lymphadenectomy. Sentinel lymph nodes (SLNs) were identified intraoperatively using a Navigator^{TM} gamma probe and the pattern of radioactive uptake was quantified postoperatively. All 1667 resected lymph nodes were examined for micrometastases using immunohistochemistry.

Results: SLNs were identified for all 57 patients (median 2, range 1–4) and were most frequently lower paraoesophageal ($n = 31$) or left gastric nodes ($n = 28$). They contained metastases ($n = 32$) or micrometastases ($n = 3$) in 35 of 37 node-positive cases and were more likely to contain tumour than other lymph nodes ($P < 0.0001$). SLN biopsy had an overall accuracy of 96 per cent (55/57) and a false-negative rate of 5 per cent (2/37). Tumour-infiltrated nodal stations received a higher proportion of radioactive uptake ($P < 0.0001$). Lower oesophageal tumours had a greater proportion of SLNs ($P = 0.018$), radioactive uptake ($P < 0.0001$) and malignant nodes ($P = 0.004$) in the mediastinum compared to cardia tumours.

Conclusions: The sentinel node concept is applicable for oesophageal adenocarcinoma and could be used to tailor the extent of lymphadenectomy. There is a close relationship between patterns of radioactive uptake and lymphatic tumour dissemination, which differ for lower oesophageal and cardia tumours.

Moynihan 02

Cavity biopsies increase the detection of residual disease following conservative surgery for breast cancer

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Aims: To examine the correlation between margin and cavity biopsy status following conservative surgery for invasive breast cancer. Does cavity biopsy increase the detection of residual disease?

Methods: In 510 patients who had conservative surgery for invasive breast cancer, four quadrant biopsies of the residual cavity were compared with the margin. Where repeat excision was performed, the presence of residual disease was recorded.

Results: The margins were positive in 102 cases and the cavity in 112, but the concordance was only 57/157 cases (36 per cent). Simultaneously, clear margins and cavities were found in 353 cases (69 per cent).

	Margin (+ve) and cavity (+ve)	Margin (+ve)	Cavity (+ve)
Ductal	34	38	48
Residual disease on re-excision	14/22	0/10	4/22
Lobular	23	7	7
Residual disease on re-excision	10/20	1/1	2/4

Cavity biopsy was the sole evidence of incomplete resection in 40 per cent of ductal and 19 per cent of lobular cancers. Evidence of residual disease after re-excision was found in 33 per cent of ductal and 52 per cent of lobular cancers.

Conclusion: There is poor correlation between margin status and cavity biopsy. Biopsy of the residual cavity after conservative surgery for breast cancer increases the detection of residual disease by 35 per cent.

Moynihan 03

Prospective randomized controlled trial of laparoscopic *versus* open inguinal hernia mesh repair: 5-year follow-up

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Aims: Laparoscopic hernia repair induces less pain and enables a quick return to normal activity, but no information is available on long-term benefits and complications. In this prospective randomized controlled trial, laparoscopic mesh hernia repair was compared with open-mesh hernia repair at 5 years' follow-up.

Methods: Patients who were randomized to either transabdominal preperitoneal laparoscopic (TAPP) repair under general anaesthetic or tension-free open-mesh hernioplasty (OPEN) under local anaesthetic were recalled after a minimum of 5 years' follow-up. Patients were required to complete a validated questionnaire, which included questions on the presence of groin pain, numbness, testicular pain, recurrence or any directly related hospital admission. An objective clinical assessment of all patients was then undertaken by one of three independent assessors.

Results: A total of 241 patients were reviewed (119 OPEN; 122 TAPP). Mean patient follow-up was 5.8 years. Paraesthesia was more frequently observed with OPEN than with TAPP (30 per cent *versus* 2 per cent; $P < 0.001$). Groin pain was present in 13 per cent of the patients in the OPEN group and 8 per cent of patients in the TAPP group. Severe pain (pain analogue scores over 50 per cent) was observed in only two patients, both of whom underwent OPEN repair. No statistically significant difference between TAPP and OPEN repairs was observed in the incidence of testicular pain (2 per cent *versus* 2 per cent), recurrence (0 per cent *versus* 2 per cent) and contralateral hernia (5 per cent *versus* 1 per cent).

Conclusions: Open and laparoscopic mesh hernia repairs are both associated with good long-term results. Laparoscopic hernia repair has significant long-term clinical advantages.

Moynihan 04

Optimization of surgical care: a prospective randomized trial

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Aims: To investigate the effects of a multimodal rehabilitation package on physical, psychological and clinical outcome in patients after bowel resection compared with a traditional postoperative regimen.

Methods: Patients requiring elective right and left hemicolectomy were randomized to receive the 'optimization package' (see Table) or the standard hospital pre- and postoperative regimen.

Preoperative	Intraoperative	Postoperative
Written and oral information	High inspired oxygen (80%)	Epidural analgesia
Preoperative assessment	Combined epidural/GA	Early oral nutrition
Probiotics	Transverse incision	Enforced mobilization
Carbohydrate loading		Early discharge
No bowel preparation		

Outcome measures of physical function were recorded preoperatively and on days 1, 7 and 30.

Results: Twenty-three patients were randomized, 13 to optimization with 10 controls. The groups were similar in terms of age (62 years *versus* 69 years), sex (M:F = 5:8 *versus* M:F = 3:7), POSSUM scores (25 *versus* 26) and ASA grades. Grip strength was significantly maintained at 24 h in the optimization group ($P = 0.03$). The time to resume a normal hospital diet was decreased (48 h *versus* 74 h; $P < 0.001$). Pain and fatigue scores were significantly reduced in the optimization group on days 1 and 7 ($P < 0.05$). Cardiopulmonary complications were lower in the optimization group (1/13 (8 per cent) *versus* 4/10 (40 per cent); $P = 0.17$). Median length of hospital stay was 3 days (2–7) in the optimization group compared to 7 days (3–10) in controls ($P = 0.001$).

Conclusion: Optimization of surgical care significantly improves patient's physical and psychological function in the early postoperative period and facilitates early hospital discharge.

Moynihan 05

Clopidogrel reduces thromboembolism after carotid endarterectomy: a randomized trial

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Aims: Postoperative thromboembolic stroke affects 2–3 per cent of patients undergoing carotid endarterectomy (CEA) and is preceded by 1–2 h of increasing embolization. The hypothesis underlying the current study was that preoperative administration of a platelet ADP antagonist (clopidogrel) would reduce embolization and was based on previous studies showing that the platelets of patients with the highest rates of postoperative embolization had increased platelet reactivity to ADP.

Methods: Total 100 CEA patients on routine aspirin therapy (75 mg) with an accessible TCD window were randomized to 75 mg clopidogrel ($n = 47$) or placebo ($n = 53$) the night before surgery. Platelet function was assessed by flow cytometry. The number of emboli detected by transcranial Doppler within 3 h of CEA was independently quantified. Time taken from restoration of flow to skin closure was used as an indirect measure of the time to secure haemostasis.

Results: Clopidogrel significantly reduced platelet activation in response to ADP ($P < 0.05$) whilst conferring a fourfold reduction in the number of patients with >10 emboli in the postoperative period (OR 4.4, 95 per cent CI: 1.4–14.4; $P < 0.008$). However, the time from flow restoration to skin closure was >40 min in 30 per cent of clopidogrel patients compared with 8 per cent of controls ($P < 0.004$).

Conclusions: This is the first study to show that a CEA patient's postoperative thromboembolic potential can be significantly reduced by preoperative pharmacotherapy. It remains to be seen whether a lower dose of clopidogrel will confer a similar reduction in embolization whilst reducing the time to achieve haemostasis.

Moynihan 06

Gastro-oesophageal cancer and the 2-week wait: do the protocols work?

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Aims: To establish the validity of national referral criteria (DOH guidelines) for gastro-oesophageal cancer and adherence to these criteria by primary healthcare physicians.

Methods: A prospective audit of the outcome of referrals made to our centre was performed. A standard referral form was provided to local general practitioners that records criteria for direct assess endoscopic referral and allows classification as urgent or nonurgent. Our unit uses a computer algorithm to reclassify priority using the criteria supplied on the referral form. All referrals categorized as urgent by their general practitioners were given an appointment within 2 weeks. General practitioners' classification was compared with that of the computer algorithm.

Results: During the 10-month period of our audit, 1169 upper GI endoscopies were performed following open access referral.

GP/DOH guidelines	Total in group	No. new cancer cases	Incidence (%)
Nonurgent/nonurgent	253	1	0.4
Nonurgent/urgent	625	17	2.7
Urgent/nonurgent	62	3	4.8
Urgent/urgent	229	21	9.2
Total	1169	42	3.6

Of the referrals received by our unit's primary healthcare physicians incorrectly coded 58.7 per cent of cases. Prioritization using the guidelines algorithm identified 38 of 42 cancers during this period. Data entry errors resulted in two of the three malignancies identified in the urgent/nonurgent group being incorrectly classified. Correct input would have increased pick up in the urgent/urgent group from 9.2 to 10 per cent.

Conclusions: Despite initial scepticism, adherence to the national criteria for urgent endoscopic referral is associated with a high incidence of diagnosis of malignancy. This study highlights the benefit to the patient of correct classification.

Moynihan 07

Short-course preoperative radiotherapy is associated with increased severity of faecal incontinence after anterior resection of the rectum

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Aims: To determine the contribution of total mesorectal excision (TME), short-course preoperative radiotherapy (SCRT), anastomotic level and other putative contributory factors to the incidence of faecal incontinence after anterior resection of the rectum.

Methods: Survivors of anterior resection performed between February 1996 and February 2001, with a functioning anorectal anastomosis, were asked to complete a telephone questionnaire regarding their current bowel habit. Faecal incontinence was scored using the St Mark's incontinence score.

Results: The median age of 124 patients who completed the questionnaire was 76 years. A total of 104 patients had neoplastic disease. Sixty-six patients (53 per cent) exhibited some degree of incontinence, median St Marks' score 6 and interquartile range of 3–10. There was a significant association between anastomotic level, and the St Mark's score ($P < 0.0001$, linear regression). The table shows the effect of gender, SCRT and anastomotic leak on incontinence scores.

	Median St Mark's score (interquartile range)	Significance (P)*
Male ($n = 55$)	3 (0–9)	0.047
Female ($n = 69$)	0 (0–5)	
SCRT ($n = 50$)	4 (0–10)	0.0014
No SCRT ($n = 74$)	0 (0–4)	
Anastomosis		
Leak ($n = 8$)	8 (3–15)	0.038
No leak ($n = 116$)	1 (0–6)	

*Mann–Whitney U-test.

Age, splenic flexure mobilization, TME, anastomotic configuration, or use of a temporary stoma had no detectable independent effect on incontinence scores.
Conclusions: Poor functional outcome following anterior resection was associated with a low anastomosis or an anastomotic leak. The finding that SCRT was an independent predictor of postoperative incontinence, regardless of anastomotic level, has not previously been reported, highlighting the need for stringent patient selection for this treatment modality

Moynihan 08

CT colonography for the detection of colorectal disease

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Introduction: CT colonography (CTC) is the new noninvasive test for the investigation of colonic disease. Introduction of multislice CT technology has resulted in increased spatial, temporal resolution with improved 3D virtual images and provides a 1.25-mm Z-plane resolution of the entire colon in a single breath-hold. The aim of the study was to compare multislice CTC (MCTC) to colonoscopy in the detection of colorectal disease.

Methods: One hundred and four patients, 57 males, median age 69 years (29–83) with symptoms of colorectal disease were studied prospectively. All patients underwent MCTC and colonoscopy. Routine CT evaluation used real-time multiplanar reformatting with virtual flythroughs performed only in problem cases.

Results: In 31 patients, colonoscopy and MCTC were normal. Colonoscopy identified 42 colorectal cancers, 45 polyps, 22 patients with diverticulosis. MCTC identified 41 colorectal cancers and missed one cancer in a diverticular stricture (sensitivity: 98 per cent; specificity: 100 per cent), all 12 polyps measuring ≥ 10 mm in diameter (sensitivity: 100 per cent), 6 of the 7 measuring 6–9 mm (sensitivity: 86 per cent) and 9 of the 26 polyps which measured 5 mm (sensitivity: 45 per cent). Colonoscopy was false positive for six polyps. MCTC was false positive for two polyps and detected a 15-mm polyp missed at colonoscopy. MCTC identified 14 of the 22 patients with diverticulosis.

Conclusions: The results show that MCTC and colonoscopy are comparable in the detection of colorectal cancers and polyps (>5 mm). Polyp detection using multislice CTC has improved when compared to best published results using spiral CT (polyps ≥ 10 mm: 100 per cent *versus* 91 per cent, 6–9 mm: 86 per cent *versus* 82 per cent).

SARS: Vascular and Biology 01–05

Thursday, 23 May 09.00–10.15

Vascular and Biology 01

Endothelin-1 promotes angiogenesis in human microvascular endothelial cells acting in synergy with VEGF and via the ET(B) receptor

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Background: Endothelin-1 (ET-1) is a 21 amino acid peptide with vasoconstrictor and mitogenic properties. We observed that ET-1 is expressed around areas of angiogenesis in ischaemic skeletal muscle, and sought to determine whether ET-1 provides an angiogenic stimulus to human microvascular cells.
Methods: Human dermal microvascular cells (HMEC-1) were grown on growth factor reduced Matrigel, with or without ET-1 at various concentrations. Synergy with the angiogenic factor vascular endothelial growth factor (VEGF) was sought by co-adding ET-1 with VEGF. In addition, we measured the effects of selective inhibitors of the ET-1 receptor. Angiogenesis was measured after 24-h treatment by visual analysis of microvessel growth and by scanning the mean optical density of images of the angiogenic field.
Results: ET-1 enhanced angiogenesis in the presence of VEGF ($P < 0.02$). The ET(B) receptor antagonist BQ788 suppressed angiogenesis below basal levels ($P < 0.04$), whereas the ET(A) receptor antagonist had no effect. Microvascular cells were found to release ET-1 when grown on Matrigel, with enhancement following treatment with VEGF.
Conclusions: These data suggest that in human microvascular cells, ET-1 is an important autocrine growth factor, acting downstream and in concert with VEGF to stimulate angiogenesis. We suggest that the mitogenic properties of ET-1 are ET(B) mediated, unlike the effects on vascular tone, which are ET(A) mediated. The separation of these effects might provide benefit to patients with peripheral vascular disease, where selective ET(A) receptor antagonists could promote vasodilatation whilst preserving the ET(B) mediated angiogenic effects.

Vascular and Biology 02

Heparin blocks stromal derived factor-1 induced migration via CXCR4 in breast cancer cells

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Background: The chemokine receptor CXCR4 is linked to chemokine-mediated metastasis in breast cancer. The breast cancer microenvironment is rich in cytokines, known to have a regulatory effect on CXCR4. We examined the inflammatory cytokine regulation of CXCR4 surface expression and its effect on migration in MCF-7 and MDA MB231 cells. As heparin competitively antagonizes chemokines we evaluated its potential to block migration of these cell lines towards the chemokine SDF-1.
Methods: Expression of CXCR4 was analysed by flow cytometry (R & D Mab 172) before and after stimulation with TNF-α or IFN-γ. The effects of TNF-α and heparin on migration were examined by chemotaxis assays.
Results: TNF-α significantly reduced CXCR4 expression in both cell lines after 24 h (MCF-7: 83 per cent*; MDA MB231: 89 per cent*). In MCF-7 cells IFN-γ reduced expression (97 per cent*) by 24 h; a bi-phasic response was observed for MDA MB 231 cells, with a maximal reduction occurring after 16 h (83 per cent*), returning to unstimulated levels by 24 h. The reduction in CXCR4 expression by TNF-α was consistent with reduced migration towards SDF-1 (MCF-7 by 80 per cent*, MDA MB231 by 94 per cent*). Heparin also inhibited chemotaxis of MCF-7 (at 250 μg mL^{-1*}) and MDA MB231 (at 100 μg mL^{-1} and 250 μg mL^{-1*}).
Conclusions: Regulation of the CXCR4 by proinflammatory cytokines alters the ability of breast cancer cells to migrate towards SDF-1. Heparin significantly reduces SDF-1 induced breast cancer cell migration, suggesting a potential therapeutic action in patients with breast cancer.
Statistics: Student's one-tailed t-test. ($^*P < 0.05$) (TNF-α: tumour necrosis factor alpha, IFN-γ: interferon gamma, SDF-1: stromal derived factor-1)

Vascular and Biology 03

Quantitative PCR analysis of hepatocyte growth factor (HGF), its receptor (c-Met) and HGF inhibitors in breast cancer

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Background: Hepatocyte growth factor (HGF) elicits a number of functions that are tumourigenic and known to enhance the metastatic potential of tumour cells. HGF is produced as pro-HGF and requires proteolytic activation, by HGF activator, to evoke a biological response. The HGF inhibitors, HAI-1 and HAI-2, suppress the conversion of pro-HGF, through their interaction with HGF activator. This study quantitated the expression of HGF, its receptor and its inhibitors in breast cancer.
Methods: Breast cancer tissues from patients ($n = 97$) were obtained with background normal tissues. RNA was extracted from these tissues, and HGF, c-Met, HAI-1 and HAI-2 expression was quantified using a real-time quantitative PCR (RTQ-PCR) techniques.
Results: Levels of HGF and its receptor were found to be significantly higher in breast cancer than normal background tissues. The level of HAI-1 and 2 was also seen to be higher in tumour tissues. The mean results (copy number mL^{-1}) are given in the Table below:

Gene of interest	Background tissue	Cancer tissue	P-value
HGF	5.2	48.8	$P < 0.01$
c-Met	9339.4	22155.5	$P < 0.05$
HAI-1	450.3	960.2	$P < 0.05$
HAI-2	114.7	438.7	$P < 0.01$

In addition, patients with progressive diseases had a higher level of HGF (62.7 copies mL^{-1}), than those with stable disease (43.8 copies mL^{-1}), over a 5-year follow-up period. Furthermore, tumour tissues from node-positive patients expressed lower HAI-2 levels (341.3 copies mL^{-1}), than the node-negative breast cancer tissues (1021.5 copies mL^{-1}).
Conclusions: This study has shown that the quantity of HGF, c-Met, HAI-1 and HAI-2 expressed in breast cancer tissues was significantly higher than that of background breast samples, and that the level of HGF is associated with progressive disease.

Vascular and Biology 04

Stabilization of breast cancer microvessels – A mechanism for tumour inhibition induced by angiopoietin-1

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Background: Angiopoietin-1 (Ang-1) functions physiologically to promote angiogenesis by inducing vascular branching and smooth muscle recruitment. However, we have previously reported to this Society that in an experimental breast cancer model, over-expression of Ang-1 led to tumour inhibition and that expression of Ang-1 in clinical breast cancer specimens was infrequent. We now report a morphological analysis of the tumour microvessels in this model in an attempt to ascertain a mechanism for tumour inhibition.
Methods: Ang-1 over-expressing and vector-transfected xenograft tumours used in previous tumourigenicity assays were fixed routinely and sectioned. Endothelial cells and smooth muscle cells were immuno-stained with monoclonal antibodies to CD-31 and smooth muscle actin, visualized with confocal fluorescence microscopy and quantitated with image analysis. Tumours were also immuno-stained for markers of proliferation and apoptosis.

Results: Ang-1 expressing tumours displayed striking differences in microvascular morphology at the leading edge of the tumour when compared with controls. Although the number of microvessels was not altered, the peripheral vessels in the Ang-1 tumours were much less ectatic than the dilated immature tumour vessels seen in controls. They were surrounded by a closely adherent continuous layer of smooth muscle cells, absent in controls. In these vessels the ratio of smooth muscle cells to endothelial cells was increased threefold ($P < 0.01$) and luminal diameter decreased twofold ($P < 0.05$) compared with controls. The rates of tumour cell proliferation and apoptosis were decreased twofold ($P < 0.001$) compared with controls.

Conclusions: Ang-1 over-expression induced stabilization of microvessels at the tumour periphery, which resulted in a decreased rate of tumour growth.

Vascular and Biology 05

17-β-Oestradiol decreases the expression of occludin and transendothelial resistance (TER) and increase paracellular permeability in human vascular endothelial cells

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Background: Tight junctions govern the permeability of endothelial and epithelial cells. Changes in tight junction function are thus an early and key event in cancer metastasis and tissue permeability. This study sought to determine the role of oestrogen in the regulation of tight junctions and expression of occludin in endothelial cells.

Methods: Human vascular endothelial cell line was incubated with 17-β-oestradiol at different concentrations (from 10^{-11} M to 10^{-7} M) over 1–24 h. Expression of occludin mRNA was determined using RT-PCR, and change of occludin protein using Western blotting. Transendothelial resistance (TER) was measured with an EVOM, and transendothelial cell permeability was determined using fluorescence labelled dextran (FITC-dextran 10) with a multi-channel fluorescence reader.

Results: 17-β-oestradiol reduced the expression of occludin mRNA in a time and concentration dependent manner with an obvious effect starting from 4 h. Reduced level of occludin protein was similarly seen after treatment with 17-β-oestradiol. Incubation of HECV with 17-β-oestradiol resulted in an increase in paracellular permeability by 9.9 ± 8.9 per cent at 10^{-10} M ($P > 0.05$ *versus* control), 42.1 ± 15.2 per cent at 10^{-9} M ($P < 0.05$ *versus* control), and 40.1 ± 22.4 per cent at 10^{-8} M ($P < 0.05$, *versus* control). A decrease in the transendothelial cell resistance (TER) was seen with oestradiol (a reduction by 18.6 ± 16.6 per cent ($P > 0.05$ *versus* control), 31.5 ± 10.6 per cent ($P < 0.01$), or 44.4 ± 18.4 per cent ($P < 0.01$), at concentration 10^{-10}, 10^{-9}, 10^{-8} M, respectively.

Conclusions: This study shows a perturbation of tight junction functions in endothelial cells by oestrogen, which may have implications in the aetiology of mastalgia and vascular spread of breast cancer.

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SARS: Upper GI 01–05

Thursday, 23 May 09.00–10.15

Upper GI 01

Nuclear factor-κB activation in Barrett's oesophagus: a marker of disease progression?

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Aims: A molecular understanding of Barrett's tumourigenesis may impact on treatment, tumour prevention and surveillance policies. Nuclear factor-κB regulates several cellular genes that participate in the inflammatory and immune responses, and this study examined NF-κB in the spectrum of Barrett's disease.
Methods: Fifty-one patients were studied, 26 with Barrett's oesophagus and 35 with Barrett's associated adenocarcinoma. Electromobility shift assay measured NF-κB expression in nuclear extracts of fresh tissue tumour samples and Barrett's biopsies. Only patients with macroscopic evidence of Barrett's greater than 3 cm were included. All Barrett's patients had samples of non-Barrett's oesophagus analysed as controls and completed a detailed reflux questionnaire. Correlation was also made with DeMeester scores.
Results: Increased NF-κB activity was observed in 16/26 (61.5 per cent) patients in Barrett's epithelium compared with samples of squamous epithelium above the Barrett's segment. All Barrett's patients with dysplasia ($N = 5$) showed significantly increased NF-κB expression. The expression of NF-κB did not correlate with symptomatology, length of Barrett's, DeMeester scores or use of proton pump inhibitors. Twenty-eight of 35 (80 per cent) patients with oesophageal adenocarcinoma showed highly elevated NF-κB expression, and this correlated with late stage of disease in these patients. NF-κB was significantly decreased in response to neoadjuvant chemotherapy and radiation therapy.
Conclusions: These data show for the first time that patients with Barrett's oesophagus have progressive increase of NF-κB expression from metaplasia through dysplasia and adenocarcinoma. NF-κB is a central regulator of inflammation and tumourigenesis, and further understanding of factors regulating NF-κB activation may offer prospects for novel anti-inflammatory and antitumour therapies in patients with Barrett's oesophagus.

Upper GI 02

Down-regulation of hepatic function, the acute phase response and severe gallstone pancreatitis

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Background: Impairment in hepatocellular function may contribute to the inability to replenish cellular glutathione, the depletion of which has been demonstrated to increase susceptibility to inflammatory stress. We sought to determine if severe acute pancreatitis (AP) is associated with down-regulation of glutathione metabolism by observing the profile of ALT (transulfuration pathway) and γ-glutamyl transpeptidase in patients with AP of biliary origin.
Methods: Venous blood samples taken at 24, 48, and 72 h from the onset of pain were analysed for ALT, γ-GT, alkaline phosphatase (ALP), and bilirubin, and correlated with (1) clinical severity (Atlanta criteria), and (2) the positive acute phase protein response (CRP).
Results: In total, 85 patients (severe 25) were recruited. In a severe attack, ALT, γ-GT and ALP but not bilirubin, were persistently and significantly lower than a mild attack (see table). Plasma ALT strongly correlated with γ-GT (24 h: $r = 0.52$, $P < 0.001$), and inversely with CRP (24 h: $r = -0.34$, $P = 0.004$).

Time		Mild (60)	Severe (25)	*P*-value
24 h	ALT	123 (15–1515)	80 (6–397)	<0.001
	ALP	372 (86–1240)	182 (27–48)	0.007
	γ-GT	240 (15–1504)	72 (42–506)	0.04
72 h	ALT	103 (7–859)	42 (12–155)	<0.001
	ALP	302 (108–184)	189 (23–515)	0.004
	γ-GT	196 (15–1515)	81 (6–397)	0.03

Conclusions: Depletion of circulating ALT and γ-GT in severe disease is likely to be secondary to a down-regulation of hepatic function, and may adversely contribute to the depletion of cellular glutathione.

Upper GI 03

Physical activity level in patients with pancreatic cancer cachexia

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Background: Physical activity level (PAL) has commonly been measured in healthy subjects. It was hypothesized that PAL would be reduced in patients with pancreatic cancer cachexia and could be increased by consuming an oral nutrition supplement enriched with the anticatabolic *n*-3 fatty acid eicosapentanoic acid (EPA).
Methods: Total energy expenditure (TEE, kcal day^{-1}) was measured over a 14-day period in 24 patients with pancreatic cancer cachexia using the doubly labelled water (^2H_2O, $H_2{}^{18}O$) technique. Resting energy expenditure (REE, kcal day^{-1}) was measured by indirect calorimetry. PAL = TEE/REE. Three-day diet diaries were completed to calculate calorie (kcal day^{-1}) and protein (g day^{-1}) ingestion. The patients were then randomized to consume two cans of nutrition supplement day^{-1} (containing 600 kcal, 32 g protein, ±EPA) for 8 weeks. PAL was remeasured between weeks 6–8. Baseline results were compared with predicted (P) values. Data: median (minimum–maximum), compared by Wilcoxon rank test.
Results: At baseline, not only were the patients hypermetabolic (REE > PREE, 1346 [994–1665] *versus* 1239 [985–1611], $P = 0.001$), but their TEE was reduced (TEE < PTEE, 1673 [1082–2459] *versus* 1858 [1478–2417], $P = 0.033$), leading to reduced PAL (PAL < PPAL, 1.18 [1.00–1.62] *versus* 1.50 [1.50–1.50], $P < 0.001$). Consumption of EPA-enriched nutrition supplements resulted in increased calorie (1391 [990–2378] to 1837 [1751–2729], $P = 0.018$) and protein (45 [37–86] to 85 [54–105], $P = 0.018$) intake. This was associated with a rise in PAL (1.32 [1.00–1.62] to 1.38 [1.19–2.01], $P = 0.018$). No significant changes occurred in those consuming placebo (minus EPA) supplements.
Conclusions: For the first time we have demonstrated that an objective measure of physical functioning (PAL) is reduced in patients with pancreatic cancer cachexia. Consumption of nutrition supplements enhanced with EPA was associated with an increase in PAL.

Upper GI 04

Outcome prediction in oesophagogastric surgery – the role of artificial neural networks in predicting individual risk

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Background: The prediction of individual patient risk has important implications in the process of informed consent. As there are no predictive indices that objectively quantify operative risk at the patient level, we compared the performance of artificial neural networks (ANN) with hierarchical logistic regression (HLR) in predicting outcomes in oesophagogastric surgery

Methods: POSSUM variables were collected from 505 patients undergoing elective ($n = 339$, 67 1 per cent) or emergency ($n = 43$, 32.9 per cent) oesophagogastric surgery in five UK hospitals between 1995 and 1999 Back-propagation ANN models were trained using genetic algorithms (18:4 6·1 network topology; input-hidden χ^2 output nodes) and a 60 15.25 per cent split sample-validation-test technique adopted for model evaluation and comparison with the HLR equivalent Bayesian analysis was used to assess model validity.

Results: The ANN completed training following 18 generations with final mean squared errors of 0.056 and 0 065 in the development and test sets HLR models performed better in group statistics. The ANN model offered better discrimination between high-risk and low-risk patients as compared to the HLR model (area under the ROC curve = 0 854 *versus* 0.838 development set, 0.937 *versus* 0 866 test set) Based on 9 3 per cent overall mortality (prior odds = 0 101) the likelihood ratios for a positive test at 10, 20, 30, 40 and 50 per cent probability of death were 1.1, 2.5, 4.3 and 10 2 for the ANN model

Conclusion: The ANN were significantly better in individualizing outcomes whereas statistical techniques were best suited for evaluating group outcomes With additional training, ANN may become attractive adjuncts to clinical management and decision-making process

Upper GI 05

T-cell adhesion and cytolysis of pancreatic cancer cells: a role for E-cadherin in immunotherapy?

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Background: Adhesive interactions between pancreatic carcinoma and cytotoxic T cells may be crucial to an effective anti-cancer response CD103 expressed on T lymphocytes is the only molecule that heterotypically binds to E-cadherin, an epithelial cell adhesion molecule characteristically involved in homotypic epithelial cell–cell adhesion The aim of this study was to show that loss of E-cadherin expression by pancreatic cancer cells, which is observed during the metastatic process, may occur as a result of the adhesion and cytolysis of cancer cells by T cells

Methods: E-cadherin negative pancreatic carcinoma cells were transfected with a plasmid vector coding for E-cadherin Peripheral blood lymphocytes were then isolated and stimulated with transforming growth factor β_1 (TGF-β_1) to express CD103 The adhesion and cytotoxic potential of these lymphocytes against pancreatic cancer cell targets were assessed by flow cytometric adhesion and standard chromium release cytotoxicity assays.

Results: CD103 expressing T lymphocytes showed significantly increased adhesion to E-cadherin transfected carcinoma cells (5 64 1) compared to wild-type pancreatic cells (1.47.1, $P < 0.0001$) Adhesion was significantly reduced in the presence of anti-CD103 blockade (2 25 1, $P < 0.001$) CD103 expressing T lymphocytes showed significantly increased cytolysis of E-cadherin transfected carcinoma cells compared to wild-type pancreatic cancer cells (18 per cent *versus* 39 3 per cent, $P < 0.001$) Cytolysis was significantly reduced in the presence of anti-CD103 blockade (17 8 per cent *versus* 38.5 per cent, $P < 0\ 001$)

Conclusions: T-cell adhesion to and cytolysis of pancreatic carcinoma cells appear to be dependent on the CD103–E-cadherin interaction Reduced or lost expression of E-cadherin in pancreatic carcinoma may therefore be part of the process whereby cancer cells escape T-cell destruction and may represent scope for immunotherapy

SARS: Transplantation 01–05

Thursday, 23 May 09.00–10.15

Transplantation 01

Addition of pharmacological agents to phosphate buffered sucrose (PBS140) confer improved protection against warm ischaemia in rat kidney

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We have evaluated the effect of the addition of pharmacological agents (Allopurinol, diltiazem, aspirin and glutathione) to phosphate buffered sucrose (PBS140) upon in situ warm ischaemia of rat kidney. Male Wistar rats (300–350 g)* were anaesthetized by intraperitoneal inactin (120 mg kg^{-1}). An intravenous saline infusion was given at 6 mL h^{-1} also containing 37 MBq L^{-1} of 3H inulin. Both ureters were cannulated for urine collection. After an equilibration period of 1 h urine was collected from each kidney for 1 h (control). The left kidney was then flushed with 0.5 mL of PBS140, UW or Solution I (PBS140 and pharmacological additives) at 37 °C. A clamp was then applied to the left renal pedicle. After 45 min the clamp was released and a right nephrectomy was performed. Inulin clearance and urine osmolality for each group were compared for preischaemic period (control) and 1- and 4-h postischaemia (mean ± SEM, $n = 6$). Postischaemic inulin clearance (1st hour) fell significantly in both PBS140 and UW but was higher in solution I (70, 57 and 103 per cent of preischaemic control, respectively). Inulin clearance gradually declined over the 4 h to 24 and 33 per cent of preischaemic control in PBS140 and UW and 55 per cent in solution I. The first and fourth hour urine osmolalities for solution I were 53 per cent and 91 per cent of preischaemic control. These were 41 and 54 per cent for PBS140 and 33 and 57 per cent for UW, respectively. The addition of the pharmacological agents to PBS140 provided significant advantage over PBS140 and UW in the protection against in situ warm ischaemia.

Transplantation 02

Rapamycin inhibits vascular remodelling in a rat model of allograft vasculopathy and attenuates associated changes in fibrosis associated gene expression

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Aim: To assess the effect of Rapamycin at different dosages on the expression of genes that regulate vascular remodelling and fibrosis in a rat model of allograft vasculopathy.

Methods: Lewis recipients of F344 rat thoracic to abdominal aorta transplants were administered Rapamycin or no treatment. F344 to F344 isografts served as controls. Vascular remodelling and ECM accumulation (Sirius Red) at 16 weeks post transplant were measured by computerized histomorphometry of aortic sections. Expression of fibrosis associated genes was studied by means of semiquantitative RT-PCR. Non parametric tests were used to compare different groups (Mann–Whitney).

Results: Rapamycin (0.5 mg kg^{-1} per day) inhibited intimal hyperplasia (intima media ratio 0.02 ± 0.02 *versus* 0.2 ± 0.05, $P < 0.01$), medial ECM accumulation (fibrosis) (Sirius red staining fraction 0.4 ± 0.01 *versus* 0.45 ± 0.01 mm^2, $P < 0.05$) and expansive vascular remodelling (internal elastic lamina circumference 4.0 ± 0.2 mm *versus* 4.2 ± 0.1 mm, $P < 0.05$) compared to untreated aortic allografts. This was associated with attenuation of the graft inflammatory infiltrate and a reduction in intragraft gelatinase, collagen 3 and TIMP 1 mRNA levels. At 0.25 mg kg^{-1} per day Rapamycin inhibited intimal hyperplasia (0.01 ± 0.1, $P < 0.01$) and medial ECM accumulation (0.41 ± 0.04 mm^2, $P = 0.05$) compared to no treatment but not vascular remodelling (4.1 ± 0.1, $P = 0.4$). Allografts receiving 0.25 mg kg^{-1} per day had a more severe inflammatory infiltrate and larger amounts of intragraft MMP 9 mRNA than those treated with the higher dose.

Conclusion: These data suggest that as well as the tissue response to injury, the alloimmune injury itself directly contributes to vascular remodelling in allograft vasculopathy. Rapamycin at higher, but not at lower doses, inhibited both of these pathological processes.

Transplantation 03

Depression of hepatic expression and serum levels of ICAM-1 and VCAM-1 during warm ischaemia reperfusion by intraportal infusion of prostaglandin E$_1$

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Background: Prostaglandin E$_1$ (PgE$_1$) may have a role in ameliorating hepatic ischaemia–reperfusion injury (IRI). Metabolism occurs after intravenous administration of PgE$_1$ and limits its efficacy. This study has investigated the cytoprotective effect of PgE$_1$ administered intraportally. Since leucocyte–endothelial cell adhesion has been strongly implicated in IRI, we also studied intracellular adhesion molcule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) expression to assess if PgE$_1$ acted through this pathway.

Methods: Under general anaesthesia hepatic ischaemia was induced in 20 canine livers by 60 min of in situ inflow occlusion with continuous intraportal infusion of PgE$_1$ 0.02 µg kg^{-1} per min lactated Ringers solution ($n = 10$) or equal volumes of lactated Ringers solution (controls, $n = 10$). The infusion started 15 min before ischaemia and continued during the ischaemic phase and 30 min after reperfusion. Hepatic venous blood and liver biopsies were taken at baseline, 60-min ischaemia and 30-min reperfusion. The blood samples were assessed for liver enzymes, TNF-α, IL-1β, serum ICAM-1 (sICAM-1) and VCAM-1 (sVCAM-1), while the liver biopsies were assessed histologically for IRI and immunohistochemically for ICAM-1 and VCAM-1 expression.

Results: PgE$_1$ treatment significantly reduced the rise in AST, ALT, TNF-α, sICAM-1 and sVCAM-1 following ischaemia and reperfusion ($P < 0.05$). It reduced hepatocyte necrosis and sinusoidal congestion. However, it did not reduce IL-1β. These changes were associated with significant down-regulation of ICAM-1 and VCAM-1 ($P < 0.05$).

Conclusion: Intraportal PgE$_1$ infusion ameliorates IRI, inhibits ICAM-1 and VCAM-1 expression and TNF-α secretion. One of the mechanisms of cytoprotection may be through ICAM-1 and VCAM-1 inhibition.

Transplantation 04

Effects of donor source on signalling and pro-fibrotic mRNA expression in pre-perfusion renal transplant biopsies

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Rat studies have indicated that rapid, irreversible CNS injury with continued perfusion may increase donated organs' susceptibility to host immune responses, thus partly explaining the difference between cadaveric and living transplant success rates. In this study, mRNA expression from cadaveric renal donors was compared to that from living donors, and also to that from asystolic donors. Biopsies were taken from renal transplants (10 living, 10 conventional cadaveric, 7 asystolic donors) before reperfusion, while on ice. Complementary DNA to glomerular mRNA was synthesized, amplified and measured by RT-PCR-ELISA. Cadaveric donor glomeruli expressed less TGF-β ($P = 0.043$) and heat shock protein (HSP)-70 mRNA ($P = 0.009$), and more tissue inhibitor of metalloproteinases (TIMP)-1 mRNA ($P < 0.001$) than living donor glomeruli. They also expressed less HSP-70 mRNA ($P = 0.025$), and more TIMP-1 ($P = 0.002$) and TIMP-2 ($P = 0.010$) mRNA than asystolic glomeruli. Asystolic glomeruli expressed less TIMP-2 mRNA ($P = 0.025$) than living donor glomeruli. The results fit the hypothesis that injury with brain death and continued perfusion (cadaveric donation) does not result in a localized release of protective stress proteins, while low-impact injury alone (living donation) does. Injury with brain death but no perfusion (asystolic donation) also resulted in the localized release of one of the proteins. This again fits the hypothesis – asystolic donors would not release 'susceptibility' factors, because the cessation of organ perfusion preceded brain death. Our data suggest that the 'susceptibility' factors may inhibit repair, as well as induce the immune responses already suggested.

Transplantation 05

TGF-β and the development of chronic graft nephropathy: relative roles of gene, message and protein in 40 cadaveric renal transplant recipients

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Aims: The profibrotic cytokine transforming growth factor beta (TGF-β1) is believed to play a key role in the development of chronic graft nephropathy (CGN); however, the aetiopathological evidence for this relationship requires clarification. The aim of this study was to determine the donor and recipient TGF-β1 genotypes and to quantify sequential changes in TGF-β1 message and protein expression in relation to the development of CGN.

Methods: Polymorphisms in the promotor region (−800, −509, aa10) of the TGF-β gene were determined using PCR-SSP in 40 donor/recipient pairings. Protocol renal biopsies (0, 3, 6 and 12 months) were subjected to histological examination (Banff grading) and real-time quantitative PCR. Plasma TGF-β levels (total and active) were determined using a commercially available ELISA.

Results: Statistically significant ($P < 0.05$) stepwise increases were observed for plasma total TGF-β levels and TGF-β mRNA compared to baseline at 3, 6 and 12 months. The plasma TGF-β1 was higher in patients with CGN at all assessment points and CGN appeared to be linked to the presence of the PP polymorphism at amino acid 10 in the donor DNA.

Conclusions: TGF-β1 is up-regulated at protein and mRNA levels during the first year following cadaveric renal transplantation and the effect of 'high producer' gene polymorphisms may also be important. This up-regulation of TGF-β1 in plasma may provide a novel noninvasive means of identifying early fibrotic damage before it becomes clinically apparent thus allowing an opportunity for intervention and salvage of otherwise fated allografts.

SARS: Lower GI 01–05

Thursday, 23 May 10.45–12.00

Lower GI 01

Patients with left-sided colorectal cancer demonstrating microsatellite instability are at risk of developing a second cancer

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Background: Tumours from patients developing multiple colorectal cancers (CRC) demonstrate a high incidence of microsatellite instability (MSI). This does not predict the development of multiple disease, since 15 per cent of sporadic CRC also demonstrate MSI. This study aims to investigate the relationship between MSI and cancer location in predicting the risk of a second cancer.

Methods: The MSI status of 100 CRC from 49 patients who developed multiple CRC (26 metachronous and 23 synchronous) were compared with 102 patients with a single CRC. Microsatellite analysis by PCR and SSCP was performed on extracted DNA at the loci Bat25, Bat26, Bat40, D2S123, D5S346 and D17S250. Cancers were classified as high-level MSI (MSI-H) when >30 per cent of loci were unstable, low (MSI-L) when <30 per cent were unstable, or stable (MSS) if MSI was absent.

Results: The median age of those with multiple cancers was 70 years (range: 33–100) and single cancer was 72 years (range: 35–84). MSI-H was demonstrated in 11 per cent of single and 51 per cent of multiple cancers ($P < 0.001$). The incidence of MSI-H is shown below.

	Proximal colon	Distal colon
Single cancer	9/37 (24%)	2/59 (3%)
Metachronous cancer	4/12 (33%)	5/12 (42%)
Synchronous cancer	7/18 (39%)	15/29 (52%)

There was no difference in the incidence of proximal MSI-H cancers, but MSI-H cancers in the distal colon occurred 14–18 times more frequently in the multiple group ($P < 0.001$).

Conclusion: MSI-H cancers identified distal to the splenic flexure should alert clinicians to the possible existence of a synchronous lesion or subsequent development of a metachronous cancer.

Lower GI 02

Increased capsaicin receptor VR1 and related regulatory mechanisms in patients with rectal hypersensitivity and urge faecal incontinence

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Background: The presence of rectal hypersensitivity (RH) and urgency with faecal incontinence is a difficult condition to treat. The pathophysiology is unclear but may involve sensitization of visceral afferent pathways. We studied the sensory mechanisms using specific antibodies to novel ion channels and receptors.

Methods: Patients ($n = 6$) with urge faecal incontinence, RH and lowered rectal compliance underwent rectal augmentation ± dynamic graciloplasty. Full thickness biopsies taken from mid-rectum were studied by immunohistochemistry and computerized image analysis (10 random fields) using specific antibodies to the capsaicin receptor VR1 expressed by nociceptors, vanilloid receptor-like protein VRL-2, expressed by autonomic nerve fibres, neuropeptides calcitonin gene-related peptide (CGRP), substance P (SP), neurotrophic factors, nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF), NGF receptor trk A, glial markers S-100 and GFAP, and nerve marker peripherin. Control rectal tissue ($n = 12$) was obtained from patients undergoing carcinoma resection. Full ethical committee approval was granted for the study.

Results: In the study group: increased VR1 immunoreactivity in nerves in both mucosal layers ($P = 0.002$), with a parallel increase in peripherin-positive neuronal fibres ($P < 0.001$); increased GDNF ($P = 0.001$) and trk A ($P = 0.001$) positive fibres were observed. CGRP immunoreactivity was increased in submucosal ($P < 0.001$) but not myenteric ganglia. VRL-2, SP, S-100 and GFAP were unchanged.

Conclusion: This is the first study to demonstrate increased numbers of nerve fibres expressing VR1, GDNF and trk A in patients with RH and faecal urgency. Alteration of peripheral sensory processes may underlie the heightened sensation and urgency experienced by these patients.

Lower GI 03

Aberrant gene expression in colonic epithelial cells – implications for the pathogenesis of ulcerative colitis

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Background: An inappropriate response to commensal bacteria by CD4 T cells has been implicated in the pathogenesis of ulcerative colitis (UC) and it is thought that colonic epithelial cells (CEC) present the bacterial antigens to CD4 T cells. A potential CEC–CD4 T-cell interaction may involve toll-like receptors (TLR), which recognize conserved bacterial antigens. TLR are expressed on CEC. We propose that CEC influence the outcome of CD4 T-cell responses towards enteric antigen.

Methods: Colons were isolated from wild-type and IL-2-deficient ($IL\text{-}2^{-/-}$) mice which spontaneously develop colitis, similar to UC. Matched samples were obtained at 4, 7 and 9 weeks of age. These time points represent pre-onset, early and established colitis. Messenger RNA from purified CEC were reverse transcribed to produce cDNA and screened for global gene expression using DNA membrane arrays. Expression of genes encoding TLR 1–6 and mediators of TLR signalling pathways were examined using RT-PCR and Western blot.

Results: DNA arrays identified altered expression of genes in the $IL\text{-}2^{-/-}$ CEC associated with transcription factors, signalling proteins, growth factors and chemokines. These changes are being validated by RT-PCR. Comparative analysis of TLR has identified decreased expression of TLR4 and TLR5 in $IL\text{-}2^{-/-}$ CEC.

Conclusion: These results show differences in signalling pathways between wild-type and $IL\text{-}2^{-/-}$ CEC and provide evidence for CEC as functional immune cells. Alterations in TLR expression support our finding that colitis is associated with an abnormal response to specific bacterial antigen with implications for the pathogenesis of UC.

Lower GI 04

Small interfering ribonucleic acid oligonucleotides (SIRNA) targeted at *REG1α* and *REG1β* reduce growth of HRT18, a rectal cancer cell line

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Background: The expression of *REG1α* and *REG1β* transcripts in colorectal cancer are associated with a reduced 36-month survival rate following surgical resection. However, the cellular consequences of *REG1α* and *REG1β* expression are unknown.

Methods: HRT18 cells were seeded at 5000 cells per well and grown under standard conditions for 11 days. RNA was extracted from cells on a daily basis, and *REG1α* and *REG1β* transcript expression determined by reverse transcription and real-time polymerase chain reaction. siRNAs designed to target the open reading frame of *REG1α* and *REG1β* were transfected into HRT18s on day 4 and their effects on cell growth determined using the tetrazolium salt conversion assay (MTT) on day 8.

Results: HRT18 cultures underwent a linear growth phase that reached a plateau at day 8 of culture. During the linear growth phase, *REG1α* transcript expression was seen to increase by 300 per cent, peaking at day 8 and then diminishing to levels similar to those seen at day 3. siRNAs targeted at *REG1α* and *REG1β* reduced cell growth as compared to a *Luciola lateralis* luciferase-targeted siRNA control.

siRNA	Anti-*REG1α*	Anti-*REG1β*	Anti-*REG1α* and -*REG1β*
Per cent reduction in MTT optical density	29*	23*	24*

*Mann–Whitney *U*-test; gene-specific siRNA *versus* anti-luciferase control, $P=0.029$

Conclusion: *REG1α* and *REG1β* expressions are maximal whilst cells are actively proliferating, and siRNA targeting of their transcripts reduces cell growth. The association between *REG1α* and *REG1β* expression and poor prognosis in colorectal cancer may be due to the effects of these proteins on tumour cell growth.

Lower GI 05

Type-5 phosphodiesterase inhibitors significantly reduce resting anal pressures in healthy volunteers

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Background: Nitric oxide is the main inhibitory neurotransmitter in the internal anal sphincter. UK 357,903 (an inhibitor of type-5 phosphodiesterase (PDE)) prevents hydrolysis of cyclic guanosine monophosphate, enhancing the effect of nitric oxide. We investigated the effect of a single oral dose of UK 357,903 on maximal anal resting pressures (MARP) in healthy subjects.

Methods: This was a double-blind, placebo-controlled, three-way cross-over study with four treatment arms. Twelve male subjects were randomized to receive three of four treatments: single oral dose of UK 357,903 (10 and 100 mg), single dose (0.5 g) of topical 0.2 per cent glyceryl tri-nitrate (GTN) paste applied perianally or oral placebo. For each study period, MARP was recorded prior to dosing and for 2.5 h postdose. Squeeze pressures were recorded 1.25 and 2.5 h postdose.

Results: One subject withdrew consent and was excluded. The maximum change from baseline MARP was 28.7, −71.6, 3.92 and −37.6 per cent for placebo, 100 mg UK 357,903, 10 mg UK 357,903 and GTN, respectively. The difference between placebo and 100 mg UK 357,903 was significant ($P=0.001$). Squeeze pressures were unchanged. High-dose UK 357,903 caused facial flushing in two subjects, headaches in four subjects and penile erections in two subjects. GTN caused headaches in four subjects.

Conclusions: This study shows that the 100 mg UK 357,903 causes a significant reduction in MARP compared to placebo and GTN. PDE-5 inhibitors may benefit patients with internal anal sphincter hypertonia, including anal fissures.

SARS: Breast 01–05

Thursday, 23 May 10.45–12.00

Breast 01

A method of tumour androgen receptor expression in primary operable breast cancer measured by flow cytometry

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Background: The role of oestrogen and progesterone receptors in breast cancer has been extensively investigated; however, the androgen receptor has received little attention. We report a flow cytometric method for the measurement of tumour androgen receptor (AR) expression on 43 primary operable breast cancers. AR expression has been correlated with grade, lymph node status, epidermal growth factor receptor (EGFR), and oestrogen receptor alpha (ERα) also measured by flow cytometry.

Method: Biotinylated AR antibody AR441 (Dako) was titrated using the LNCaP cell line. Tumour samples were processed through mesh and the resulting suspension were fixed with 2 per cent paraformaldehyde and permeabilized with 0.025 per cent triton prior to incubation with AR antibody, streptavidin PE and 5D3-FITC. Analysis was performed on cytokeratin-positive cells by FACScan flow cytometer.

Results: Eighty per cent of tumours expressed AR. There was a significant correlation between AR and ERα expression (P-value = 0.008). No correlation was observed between AR and EGFR expression (P-value = 0.59).

	Number	AR expression, mean value (mol fluorescene cell^{-1})	P-value
Grade 1	3	1895	0.13
Grade 2	9	6275	
Grade 3	16	10702	
Lymph node negative	15	10111	0.68
	13	7246	

Conclusions: AR appears to be coexpressed with ERα, but not EGFR. The finding of increasing AR expression with increasing grade suggests that AR expression is a more aggressive marker than has hitherto been found by immunohistochemistry. These observations are supported by the previously reported findings of AR expression in 75 per cent of breast cancer metastases, and as the sole steroid receptor in 25 per cent of metastases.

Breast 02

Cyclo-oxygenase inhibitors reduce spontaneous metastases and serum VEGF in an experimental model of breast carcinoma excision

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Background: Cyclo-oxygenase-2 (COX-2) and VEGF expressions are increased in breast cancer, and surgery has been shown to increase the growth of metastatic tumours. The aim of our experiment was to investigate the effect of selective COX-2 inhibition on tumour metastases in an experimental excision model of breast cancer.

Methods: A total of 50 000 4T1 mammary carcinoma cells were injected into the left mammary fat pad of 9-week-old female BALB/c mice. On day 11, postinjection (mean tumour size = 7.4 ± 0.4 mm) mice were randomized into three groups ($n = 6$ per group) to receive daily intraperitoneal (IP) injections of the selective COX-2 inhibitor, SC-236 pre- and postexcision of primary tumour, postexcision of primary only or drug vehicle. Breast tumours were excised on day 14, and animals were sacrificed 14 days postexcision of primary.

Results: At harvest, there was a significant reduction in tumour burden, the number of lung metastases and in serum VEGF levels between the SC-236 and control group. There was no significant difference between the pre-excision- and postexcision-treated groups.

At harvest	Control	SC-236, pre-excision	SC-236, post-excision
No. pulmonary metastases	22.5 ± 5.2	$11 \pm 3.1^*$	$5 \pm 5.8^*$
Metastatic burden (lung wt/body wt)	1.05 ± 0.05	$0.86 \pm 0.05^*$	$0.86 \pm 0.05^*$
Serum VEGF (pg mL^{-1})	83.5 ± 20.9	$35 \pm 3.9^*$	$30.5 \pm 6^*$

ANOVA; nonparametric analysed with Kruskal–Wallis test; $^*P < 0.05$.

Conclusion: This data demonstrates that selective COX-2 inhibition significantly reduces the number of spontaneous metastases in an experimental excision model of breast cancer. We have also shown that this may occur secondary to a reduction in the potent angiogenic factor VEGF.

Breast 03

Lymphangiogenesis and lymph node metastasis in breast cancer

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Background: Breast cancer affects one in 10 women in the UK. No link has been demonstrated between angiogenesis and regional metastasis in breast cancer. Many cancers spread primarily via the lymphatics, yet research into lymphangiogenesis and metastasis has been limited. Using all known lymphatic markers, we quantified lymphangiogenesis by measuring mRNA expression of lymphatic markers, using quantitative polymerase chain reaction (QPCR) techniques. Furthermore, the relationship between lymphangiogenesis and axillary lymph node status was examined.

Methods: RNA was extracted and cDNA synthesized from 120 archival breast cancers and 33 background breast samples. Plasmids containing human sequences from the following markers were constructed and used as internal standards: LYVE-1, Prox1, Podoplanin, VEGF-C, VEGF-D, VEGFR-3 and VEGFR-2. The first six are lymphatic markers, whereas VEGFR-2 is a blood vessel marker. QPCR was performed on the breast cDNA and plasmids. The original concentration of each marker was quantified by reference to the plasmid standards' concentrations.

Results: Mean values for node positive, negative and background tissue for each marker (all values in copies μL^{-1}): LYVE-1: 128, 12.1, 0.3; Prox1: 6140, 4466, 1270; podoplanin: 23306, 7764, 4973; VEGF-C: 3989, 613, 270; VEGF-D: 115, 32, 12; VEGFR-3: 129, 117, 66. Most differences were statistically significant (Mann–Whitney U-test, $P < 0.05$). No differences were found between the three groups with VEGFR-2.

Conclusions: This first quantitation of lymphangiogenesis has demonstrated that lymphangiogenesis is higher in node-positive than in node-negative tumours, and the lowest in normal breast. This suggests that those cancers which overexpress lymphatic markers are more likely to metastasise to the lymphatics.

Breast 04

Sentinel node biopsy in breast cancer: a complete immunohistochemical study

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Background: To discover the true accuracy of sentinel node biopsy (SNB) by eliminating the bias that results from the more intensive histological assessment of the SNs than non-SNs.

Methods: Three hundred unselected consecutive patients with primary breast cancer (T1-3, N0-1) had sentinel biopsy followed by level III axillary clearance. SN localization was by isotope and dye in 112 patients and dye only in 188 patients All sentinel and nonsentinel nodes were treated identically by serial sectioning, followed by H&E and immunohistochemistry for cytokeratin (CAM 5 2)

Results: SN was identified in 250 (83 3 per cent), 91 9 per cent for isotope and dye compared to dye alone, 78 2 per cent ($P = 0\ 005$). A mean of 1 4 SNs and 21 8 non-SNs were retrieved. The SN predicted the status of the rest of the axilla with an accuracy of 91 2 per cent and a sensitivity of 82.3 per cent There was no difference in the sensitivity of SNB between the methods of localization used (isotope and dye: 84.6 per cent; dye alone: 81 1 per cent). Multivariate analysis of 10 patients and methodological variants demonstrated that needle localization of the tumour and high node positivity ($\geq$4 positive nodes) were the only factors associated with reduced SN identification. None of the same 10 factors affected SNB accuracy

Conclusions: In this prospective study when patients are not selected and all of the axillary nodes are cleared and treated by similar histological techniques, the accuracy and sensitivity of SNB is not as accurate as generally quoted Different methodological techniques affect the rate of identification of the SN but not its accuracy

Breast 05

The identification of drug resistance in breast cancer: a feasibility study

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Background: In an attempt to identify drug resistance in breast cancer before treatment, an *in-vitro* study is described using cells isolated from fine-needle aspirate (FNA) samples

Methods: Cells isolated from 78 patients at diagnosis were continuously exposed for 48 h and/or 96 h to four concentrations of drugs. Cell survival was measured using either the ATP or MTT (3,4,5-dimethylthiazol-2,5-diphenyl tetrazolium bromide) assays Dose–response curves and the LC_{50} values (the concentration required to kill 50 per cent of cells) were calculated Results from *in-vitro* studies were compared with age, Nottingham prognostic index (NPI) and histology but the clinical outcome is not yet mature.

Results: The ATP assay was found more suitable since it required a minimum of 10 cells per well compared to the MTT assay which requires at least 1×10^5 Fifty of the 78 (64 per cent) samples produced sufficient cells, of these 43/49 malignant samples (87 per cent) achieved a successful assay There was considerable variation in the response to cytotoxic agents tested after 48 h.

Cytotoxic agent	Median LC_{50} ($\mu g\ mL^{-1}$)	Range	n
Mafosfamide	3 37	0 14–35	43
Doxorubicin	1.34	0.18 to >10	13
5Fluorouracil	296	8 88–750	12

Patients defined as having poor prognosis according to the NPI were more likely to produce results ($P < 0.01$), the median NPI on successful assays was 3 66 (range: 2 16–6 33; $n = 32$) compared to 2.9 (range. 2.18–3.3, $n = 8$) for unsuccessful assays. There was no evidence of correlation between *in-vitro* results and prognostic indicators

Conclusions: The identification of drug resistance before adjuvant and neo-adjuvant chemotherapy for breast cancer is now a practical possibility, but correlation with the clinical outcome will be important

SARS: Upper GI and Oncology 01–05

Thursday, 23 May 10.45–12.00

Upper GI and Oncology 01

Decreased tissue beta-galactoside alpha-2,6-sialyltransferase activity in hepatocellular carcinoma – a possible cause for the formation of tumour-specific alpha-fetoprotein isoforms

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Background: Measurement of alpha-fetoprotein (AFP) is widely used in diagnosing hepatocellular carcinoma (HCC). However, moderately elevated AFP levels are also found in patients with chronic liver diseases. Separated by isoelectric focusing, AFP isoforms appearing as Band + II have been found to be specific for HCC. Recently, the Band + II isoforms have been identified to be monosialylated AFP (msAFP), which has one sialic acid residue fewer than the predominant AFP isoforms associated with liver cirrhosis. The beta-galactoside alpha-2,6-sialyltransferase (ST2,6Gal I) is the key liver enzyme to transfer terminal sialic acid to the carbohydrate side chain of AFP.

Methods: To investigate whether decrease of ST2,6Gal I expression and/or enzyme activity is the underlying cause for the formation of msAFP, serum sample, tumour and nontumour samples were collected from 19 HCC patients for the measurement of serum msAFP percentage, tissue ST2,6Gal I activity, and ST2,6Gal I mRNA.

Results: Serum msAFP percentage was negatively correlated with absolute ST2,6Gal I activity in the tumour tissue ($r = -0.423$, $P = 0.07$) and with the percentage change of enzyme activity ($r = -0.540$, $P < 0.02$). Positively correlation between the enzyme activity and the mRNA level of ST2,6Gal I was found in nontumour tissues ($r = 0.643$, $P < 0.01$), but not in tumour tissues.

Conclusion: These results suggest that decrease of ST6Gal I activity in tumours is a possible cause for the formation of HCC-specific hyposialylated AFP isoforms. In contrast to nontumour tissues, the down-regulation of the ST6Gal I activity in HCC may not be regulated at the transcription level.

Upper GI and Oncology 02

Heterogeneity of response to chemotherapy in liver tumours evaluated by the comet assay in an isolated perfused human liver

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Aims: Traditional models of studying chemotherapy efficacy do not take into account microenvironment nor do they behave like naturally occurring human tumours. An isolated human liver model with tumour was developed to provide information for overcoming barriers to drug delivery and for developing novel therapeutic strategies.

Methods: Liver specimens obtained from eight patients undergoing hemi-hepatectomy for hepatocellular carcinoma (HCC) or colorectal cancer (CRC) metastases were perfused via portal vein and hepatic artery branches (one control; three cisplatin: 23.3, 50 and 100 μM), two etoposide and two nitrogen mustard (NM; 50 and 100 μM). Chemotherapy-induced DNA damage was measured in individual tumour cells from fine needle aspiration biopsies using the Comet Assay.

Results: Thirteen tumours (median size 5 cm, range 2–17) were perfused (1 HCC, 12 CRC). Median duration of perfusion was 300 min (range 240–360), and median total perfusion flow was 300 mL min^{-1} (range 110–240). Laser Doppler imaging confirmed adequate perfusion, and livers remained viable on gross appearance, stable portal vein and hepatic artery pressures, bile production and maintenance of DNA integrity of the control. DNA damage increased linearly with cisplatin concentration (23.3, 50 and 100 μM caused 35, 45 and 60 per cent decrease in tail moment, respectively), but not with etoposide and NM. Seven of the 12 tumours demonstrated increased DNA damage superficially compared with deeper levels (three cisplatin; two etoposide and two NM).

Conclusions: Perfusion of human livers obtained during hepatic resection is a simple procedure that provides new insights into the distribution patterns of chemotherapy within tumours in an *ex vivo* model.

Upper GI and Oncology 03

Ischaemic preconditioning can prevent fatal ischaemic insult in cirrhotic liver resection

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Background: One of the major problems associated with liver resection is the development of liver failure after surgery. It was thought to be related to the ischaemic insult induced to the remnant liver at the time of the hilar inflow occlusion during liver resection. Ischaemic preconditioning, however, has been shown to be effective in other organ systems to prevent ischaemic and also ischaemic-reperfusion injury. In this study, we tested if ischaemic preconditioning can protect cirrhotic liver from ischaemic insult during liver resection.

Methods: Wistar Furth rats were induced to develop liver cirrhosis by thrice weekly injection of thioacetamide intraperitoneally for 8 weeks. Ischaemic preconditioning is induced by 10 min of hilar clamping followed by 15 min of unclamping. Rats were then randomized into two groups of either with or without ischaemic preconditioning. The rats were then subjected to either 15 or 30 min of hilar clamping followed by 60 per cent hepatectomy.

Results:

Procedures	Survival
15-in. hilar clamp	8/8 (100%)
10-in. hilar clamp + 15-in. unclamping + 15-in. hilar clamp	8/8 (100%)
30-in. hilar clamp	0/8 (0%)***
10-in. hilar clamp + 15-in. unclamping + 30-in. hilar clamp	8/8 (100%)

Conclusion: Our data suggest that ischaemic preconditioning is a useful procedure to protect the cirrhotic rats from dying after a 60 per cent liver resection. Further studies in the mechanisms of the protection would offer more information before we can apply this manoeuvre in clinical practice.

Upper GI and Oncology 04

The effects of an awareness-raising programme for patients and primary care physicians on the early detection of gastro-oesophageal cancer

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Background: Gastro-oesophageal cancer is usually detected late, when prognosis is poor. Japanese evidence suggests that increased public awareness may improve early detection rates.

Methods: We conducted a prospective controlled trial to test the efficacy of education by home letter, combined with improved specialist/GP liaison, in promoting the detection of treatable gastro-oesophageal cancer in 37 500 patients over 40. Patients were sent yearly letters for three consecutive years, advising consultation for four specific symptoms. A total of 60 500 matched controls received no intervention. Cancer cases were identified from registry data, and stage and operability from hospital records.

Results: Twenty of the 59 cancer patients in the study group (34 per cent) and 42 of 125 in the control group (34 per cent) underwent resections. Twenty-four per cent (14/59) of study group and 18 per cent (23/125) of control patients had 'curable' disease (odds ratio = 1.51, $\chi^2 = 1.164$, $P = 0.28$) In year 1 of the study, 11 of 20 (55 per cent) study group patients underwent resection compared to 10 of 38 (26 per cent) control group patients ($P = 0.045$, Fisher's exact test, odds ratio 3.4). In year 1, 8 of 20 study patients (40 per cent) had 'curable' disease compared to 10.5 per cent (4/38) control patients ($P = 0\ 0128$, Fisher's exact test, odds ratio 5 67) There was no difference in survival between the group's overall or on comparing each year of study

Conclusion: This intervention caused a transient improvement in resection rate and curability, but survival was not affected Improving the detection of curable upper-GI cancer is likely to require more complex and intensive interventions.

Upper GI and Oncology 05

Nuclear factor kappa B (NFκB) and p38 MAP kinase inhibition improves survival in endotoxin and polymicrobial models of sepsis

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Aims: NFκB and p38 MAP kinase are critical intracellular signal transduction pathways which mediate the systemic inflammatory response syndrome. Antibiotic induces bacterial lysis, also contributes to cytokine production and the inflammatory response by activating NFκB and p38 kinase. In this study, we set out to examine the effects of inhibition of p38 and NFκB translation in *in-vivo* models of sepsis

Methods: Intraperitoneal lipopolysaccharide (LPS) ($45\,\mathrm{mg\,kg^{-1}}$), tail vein injection of bacteria (*Staphlococcus* and *Salmonella*: $5 \times 10^{-8}\,\mathrm{cfu\,kg^{-1}}$) and caecal ligation and puncture (CLP) with or without antibiotics (Co-amoxyclav, $100\,\mathrm{mg\,kg^{-1}}$) were the septic models employed Animals received control, SB-202190 a p38 inhibitor or SN-50 an NFκB inhibitor (±antibiotics for CLP), and mortality was assessed by log-rank analysis. Blood was collected at different time points for cytokine analysis and splenic tissue for cytoplasmic protein extraction to assess kinase activation analysis by Western blot

Results: SB-202190 and SN-50 resulted in significant survival benefit in the LPS model ($P = 0\ 0006$) but not bacterial or CLP model ($P = 0.9$, 0 3) SB-202190 and SN-50, in combination with antibiotic, resulted in a significant survival benefit in the CLP model ($P = 0.0001$; 0 006) Circulating levels of TNF-α and IL-6 were significantly reduced at 2 h ($P = 0\ 047$, 0 036*) but not at 6 h ($P = 0.18$; 0.024) post-CLP (Table 1). Western blot demonstrated down-regulation of p38 kinase 2 h after CLP.

	TNF ($\mathrm{pg\,mL^{-1}}$)	IL-6 ($\mathrm{pg\,mL^{-1}}$)
Vehicle only	73 (±45)	118 (±42)
Vehicle and antibiotic	27 (±2)	113 (±48)
SN-50 and antibiotic	20 (±4)	92 (±25)
SB-202190 and antibiotic	13 (±1)*	27 (±2)*

Statistics. *$P < 0\ 05$, ANOVA

Conclusions: We have demonstrated that p38 and NFκB inhibition improves survival in endotoxin shock, whereas the survival benefit in polymicrobial sepsis requires coexistent antibiotic treatment. Our findings suggest that p38 and NFκB inhibition may provide novel strategies in preventing and treating sepsis in clinical practice

SARS: Breast 06–13 Thursday, 23 May 13.30–15.30

Breast 06

Investigation of systemic folate status and level of DNA damage in mononuclear cells of breast cancer patients

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Background: Folate is required for DNA synthesis, repair and methylation, and low folate status has been implicated in carcinogenesis possibly as a result of higher rate of genetic damage.

Aim: To investigate the relationship between DNA damage and folate status in breast cancer and benign breast disease control patients.

Materials and methods: Fasting blood samples from 52 histologically confirmed untreated breast cancer patients (mean age: 57 years) and 30 benign breast disease control patients (mean age: 51 years) were obtained. Mononuclear cells were isolated for genetic damage analysis using basic alkaline comet assay (which detects DNA single-strand breaks) and modified comet assay (using endonuclease III and formamidopyrimidine glycosylase (FPG) to recognize oxidatively damaged pyrimidines and purines, respectively). Results are expressed as tail moment.

Results: Results were log transformed to normalize the data and presented as geometric mean (95 per cent confidence interval).

	Cancer	Control	Significance
Red cell folate (ng mL^{-1})	339.07 (333.3–404.6)	379.5 (335.8–505.2)	0.24
Plasma homocysteine (µmol L^{-1})	11.90 (10.6–16.45)	10.1 (9.3–11.9)	0.073
Basic alkaline comet	4.30 (1.26–1.66)	0.827 (−0.43–0.08)	<0.0001
Modified comet (endo III)	5.89 (1.56–1.98)	2.36 (0.59–1.13)	<0.0001
Modified comet (FPG)	5.31 (1.46–1.88)	2.69 (0.72–1.26)	<0.0001

Conclusion: DNA damage was found to be significantly higher in mononuclear cells of breast cancer patients compared to benign breast disease control patients. Breast cancer patients tended to have lower red cell folate and higher plasma homocysteine concentrations, but these differences were not significant. A mechanistic role for low folate status in the aetiology of breast cancer is under further investigation.

Breast 07

Novel beneficial effects of anticoagulants on neoplastic processes

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Background: There is evidence that the heparins may affect tumour growth. We examined the effects of heparin and low molecular weight heparins in breast and colon cancer.

Methods:

- *Experiment 1.* Human colon cell lines SW 480, 620 and 707, and breast cancer cell lines 4T1 and MDA were incubated with medium alone, heparin, enoxaparin or tinzaparin, correlating with prophylactic to therapeutic doses. Tumour cell proliferation was assessed.
- *Experiment 2.* Balb/c mice were inoculated with 4T1 cells intravenously, and received control, heparin, enoxaparin or tinzaparin at low and high doses subcutaneously daily. Animals were killed on day 12 and the lung:body weight ratio and metastatic score were calculated.

Results:

- *Experiment 1.* High-dose tinzaparin (33.4 per cent ± 13.1) inhibited breast tumour cell proliferation when compared to enoxaparin (65.4 per cent ± 14.1) or heparin (97.6 per cent ± 12.9) ($P < 0.004$). High-dose heparin (52.9 per cent ± 18.1) inhibited colon tumour cell proliferation significantly greater than either enoxaparin (110.2 per cent ± 31.9) or tinzaparin (125.9 per cent ± 13.1) ($P = 0.003$).
- *Experiment 2.* Tumour growth was less in the low-dose tinzaparin group compared to the low-dose heparin group (lung : body weight ratio = 0.008 and 0.01, respectively; $P = 0.02$). Tumour growth was less in the high-dose tinzaparin group compared to the high-dose enoxaparin group (lung : body weight ratio = 0.008 and 0.01, respectively; $P = 0.02$; metastatic score = 2.6 and 3.9, respectively; $P = 0.02$).

Conclusions: These results indicate that tinzaparin may be the anticoagulant of choice in patients with neoplastic disease in prophylactic or therapeutic settings.

Breast 08

Taurine attenuates T-lymphocyte apoptosis induced by interleukin-2 through a Fas–Fas ligand-dependent pathway

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Background: Interleukin-2 is used in immunotherapy of melanoma and renal cell carcinoma and results in a transient reduction in circulating T-lymphocytes. The mechanism underlying this reduction in lymphocytes is unknown. *In vitro* prolonged exposure to IL-2 and costimulation of T-cells results in AICD, mediated by interaction between Fas and FasL on T-cells. Intracellular taurine levels are known to be important in regulation of T-cell death. We hypothesize that IL-2 increases Fas/FasL expression on T-cells, which results in T-cell apoptosis, and that preloading T-cells with taurine attenuates T-cell apoptosis induced by IL-2 through regulation of Fas/FasL.

Methods: T-cells were treated with anti-CD3 mAb and IL-2 to induce Fas and FasL expression. Some groups were preloaded with taurine. Fas and FasL expression, apoptosis and survival were assessed via flow cytometry.

Results:

Treatment	Fas (MCF)	FasL (% of expressing cells)	Apoptosis (%)	Survival (%)
Control	18.59	4.04	6.81	91.28
Control + taurine	16.33*	5.17	7.64	91.49
Anti-CD3 + IL-2	16.95	31.00*	34.54*	63.77*
Anti-CD3 + IL-2 + taurine	14.47*	15.80**	23.90**	74.8**
Anti-CD3 + IL-2 + rhFas:Fc	–	–	24.51	70.53
Anti-CD3 + IL-2 + taurine + rhFas:Fc	–	–	22.56	71.38

Data expressed as mean; MCF: mean channel fluorescence; **versus* control: $P < 0.05$; ***versus* anti-CD3 + IL-2: $P < 0.05$.

Conclusions: The simultaneous administration of anti-CD3 and IL-2 increases FasL expression and apoptosis of T-cells. Preloading T-cells with taurine reduces Fas and FasL expression and apoptosis induced via anti-CD3 and IL-2. IL-2 receptor levels were not altered by treatment with taurine. Treatment of T-cells with anti-CD3 and IL-2 in the presence of Fas:Fc inhibitor resulted in a similar attenuation of apoptosis compared to that observed with taurine preloading, indicating that the reduction in apoptosis attributed to taurine is dependent on the FasL pathway.

Breast 09

Pattern of expression of genes linked to epigenetic silencing in human breast cancer

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Background: A growing body of evidence now supports the hypothesis that 'epigenetic' mechanisms such as DNA methylation play an important role in neoplasia. The aim of this study was to determine the pattern of expression of multiple key cancer genes that are known to undergo epigenetic inactivation by promoter hypermethylation in breast cancer.

Methods: The expression of the tumour suppressor gene *p16*, estrogen receptor a (ERa), ERb, progesterone receptor (PR) and DNA repair genes *hMLH1* and *MGMT* were studied with immunohistochemistry in 200 breast cancers. Methylation-specific PCR was performed in a subset of 20 cancers to confirm the role of DNA methylation in the loss of expression of MGMT.

Results: The majority of breast cancers showed loss of expression of at least one gene ($n = 157$, 78.5 per cent). A strong correlation was seen between high-grade tumours and loss of expression of ERa ($P < 0.0001$), PR ($P < 0.0001$), ERb ($P < 0.0001$) and MGMT ($P = 0.04$), while loss of expression of *p16* was associated with low/intermediate-grade tumours. No loss of expression of *hMLH1* was seen in any of the tumours studied. DNA methylation was strongly associated with loss of expression of *MGMT* ($P < 0.001$).

Conclusions: The pattern of epigenetic inactivation of key cancer genes is complex with high-grade tumours showing methylation-linked silencing of certain genes with concurrent normal expression (*hMLH1*) or overexpression of other genes (*p16*). This has important implications for developing new preventive and therapeutic strategies based on epigenetic events in breast cancer.

Breast 10

In vivo complex permittivity measurements of human liver parenchyma and hepatic colorectal metastases

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Background: Microwave ablation is a technique for treating unresectable liver tumours. Accurate calculations of the interaction between microwaves and the material to be treated can only be made when that tissue's complex permittivity is known. In this study, complex permittivity of normal liver parenchyma and hepatic colorectal metastases was calculated at a frequency of 2.45 GHz.

Methods: A small microwave signal of varying frequency emitted from a computer-controlled Vector Network Analyser was passed into an open-ended coaxial sensor. The sensor was placed into tumour or normal hepatic parenchyma. Using computer software, the material's complex permittivity was calculated from the magnitude and phase of the reflected signal. For each patient, the mean of five readings from the normal liver and metastatic deposits was recorded.

Results: Successful calibrations were obtained prior to each series of readings. Data from nine patients with 11 tumours was obtained. The real part of complex permittivity was found to be consistently higher in tumour (50.27, SD: 2.49) when compared to normal parenchyma (43.28, SD: 2.18); $P < 0.0001$. Higher readings indicate the ability of tumour tissue to absorb greater microwave energy than normal parenchyma.

Conclusions: Reproducible differences in complex permittivity of tumour and normal hepatic parenchyma at 2.45 GHz were found. This data will aid the design of future microwave applicators, which will radiate energy more efficiently into tumour tissue. This will be achieved using the data gained to produce more accurate computer models of the interaction between tissue and the electromagnetic field.

Breast 11

Evaluation of 1p36 region by FISH – catching the one that almost got away!

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Background: There is considerable interest in chromosomal band 1p36, which exhibits frequent loss of heterozygosity (LOH) in a variety of human malignancies, suggesting the presence of a yet unidentified tumour suppressor gene (TSG). The faint terminal sub-bands often make cytogenetic analysis of 1p36 difficult. Small deletions at this locus may therefore be missed on analysis by conventional cytogenetics, a hypothesis that we have tested using fluorescence *in-situ* hybridization (FISH) in malignant lymphoma.

Materials and methods: The cohort consisted of 20 cases of lymphoma of various subtypes without any 1p abnormality on G-banded karyotyping. FISH was performed using human chromosome 1 paint and a bacterial artificial chromosome (BAC) probe RP4-755G5 localizing to 1p36.33, the most telomeric sub-band of 1p36. Tumours which demonstrated 1p36.33 deletions were further analysed by FISH using a second probe from the proximal 1p36 sub-band to further define the breakpoint.

Results: Seven cases of follicular lymphoma (FL), five diffuse large B-cell lymphomas (DLBCL), two Hodgkin's disease (HD), three B-cell chronic lymphocytic lymphomas (BCCLL), two T-cell and one mucosa-associated lymphoid tissue lymphoma (MALT) were analysed. FISH identified deletions at 1p36.33 in five of the 20 cases. All of these were DLBCL or FL. There was no loss of signal at the more proximal band of 1p36 in any of these cases.

Conclusions: FISH is considerably more sensitive for identifying genetic alterations than conventional cytogenetics. Deletion of the distal part of 1p36 may be a much commoner aberration than previously recognized in lymphoma.

Breast 12

Serum testosterone in women with primary operable breast cancer

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Background: The preliminary results of the IMPACT trial have shown that aromatase inhibitors are the preferred first-line hormonal treatment for advanced breast cancer. This raises the question as to the significance of circulating testosterone levels in women with breast cancer.

Method: We have measured serum testosterone and sex hormone-binding globulin (SHBG) preoperatively in women presenting with primary operable breast cancer and age-matched controls. Tumour androgen receptor (AR) expression was measured by flow cytometry on tumour biopsies from 35 patients, and serum testosterone levels were correlated with tumour expression.

Results:

Menopausal status	Number	Age, mean value	Total testosterone, mean value (ng mL^{-1})	P-value	Free androgen index[a]	P-value	Tumour AR molecules, (fluorescence cell^{-1})
Pre							
Case	13	43.5	0.64	0.09	4.2	0.41	6121
Control	12	43.2	0.46		3.4		
Post							
Case	28	56.9	0.59	0.04	2.7	0.013	6950
Control	17	56.7	0.47		4.2		

[a]([Total testosterone in nmol L^{-1}]/[SHBG in nmol L^{-1}]) × 100.

No correlation was found between serum androgen levels and tumour androgen receptor expression.

Conclusions: These results suggest that elevated circulating total testosterone has a role in the aetiology of the breast cancer, presumably by conversion to oestrogens. The finding of a significantly raised free androgen index in postmenopausal controls suggests that 'active' testosterone may be protective against the development of breast cancer. This is supported by cell line work, which has shown a growth-inhibitory affect of androgens on breast cancer proliferation.

Breast 13

Serum adrenal androgens in women with primary operable breast cancer

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R. Bliss and T.W.J. Lennard

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Background: The role of androgens in the progression and aetiology of breast cancer has resurfaced in the light of effective aromatase inhibitors. We have measured serum androstenedione, dehydroepiandrosterone sulphate (DHEAS) and cortisol in 53 pre- and postmenopausal women with primary operable breast cancer and 39 age-matched controls to examine the relationship between them.

Method: Serum was taken preoperatively from patients with primary operable breast cancer and age-matched controls recruited from the diagnostic breast clinic. Enzyme-linked immunoassays (DRG diagnostics) were used to determine serum levels of androstenedione, DHEAS and cortisol.

Results:

Menopausal status	DHEAS, mean value (ng mL^{-1})	*P*-value	Androstenedione, mean value (ng mL^{-1})	*P*-value	Cortisol, mean value (ng mL^{-1})	*P*-value	Number	Mean age
Pre								
Case	2.1	0.02*	1.54	0.38	167	0.07	17	44.8
Control	1.2		1.35		115		17	44.7
Post								
Case	1.42	0.33	2.1	0.13	156	0.13	36	58.5
Control	1.22		1.4		125		22	58.7

Conclusions: We have observed that compared to controls, adrenal androgens are elevated in patients with breast cancer. In order to examine whether levels were elevated in the patients with breast cancer due to preoperative emotional stress, we have measured serum cortisol levels. Cortisol levels were nonsignificantly raised in patients with breast cancer compared to controls; however, adrenal androgen levels were independent of cortisol levels. These findings suggest that elevated circulating adrenal androgens may have a role in the aetiology and progression of breast cancer.

SARS: Vascular 05–12

Thursday, 23 May 13.30–15.30

Vascular 05

Differential expression of selectins in a model of hindlimb ischaemia reperfusion injury (IRI)

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Aim: Leucocyte adhesion to the endothelium is implicated in the pathogenesis of IRI in several vascular beds, including skeletal muscle. The magnitude and time course of expression of adhesion molecules such as E- and P-selectin is an important and rate-limiting step in the pathogenesis of IRI.

Methods: A dual radiolabelled monoclonal antibody (mAb) technique was used to quantify E- and P-selectin expression in (a) rat hindlimbs with tourniquet-induced ischaemia for 4 h, followed by 3, 6 or 24 h of reperfusion, (b) the non-ischaemic hindlimb and (c) the small bowel in rats exposed to IRI and a sham protocol of IRI.

Results: There was no significant increase in E-selectin expression, regardless of reperfusion time when compared to sham animals or the non-ischaemic hindlimb. No E-selectin expression was detected in the small bowel of animals of either experimental or sham protocols. In contrast a significant increase in P-selectin expression was observed in the ischaemic limb 3.1, 4.3 and 1.7 ng mAb g^{-1} compared to the non-ischaemic limb 0.2, 0.1 and 0.1 ng mAb g^{-1}, at 3, 6 and 24 h, respectively. P-selectin expression was significantly raised in the small bowel at 3-h reperfusion: 0.6 ng mAb g^{-1} tissue *versus* 0.5 ng mAb g^{-1} tissue for sham.

Conclusion: Skeletal muscle IRI is associated with a significant increase in P- but not E-selectin expression in the injured hindlimb and small bowel. This differential response may be related to platelet accumulation in tissues exposed to IRI.

Vascular 06

Synergistic effects of rapamycin and tacrolimus are preferential to its combination with cyclosporin in the development of intimal hyperplasia

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Aims: Allograft vasculopathy remains the leading cause of late graft failure following heart transplantation. The antiproliferative drug sirolimus has been shown to inhibit the development of intimal hyperplasia, a central feature of allograft vasculopathy. This study aims to assess the efficacy of dual combination of sirolimus and calcineurin inhibition on the development of intimal hyperplasia, reflective of modern clinical immunosuppressive practice.

Methods: Male Sprague-Dawley rats were assigned to receive sirolimus (0.05 mg kg^{-1} per day) and either tacrolimus (0.1 mg kg^{-1} per day) or cyclosporin (5 mg kg^{-1} per day) and compared to an untreated control group. All animals underwent left common carotid artery balloon angioplasty and were explanted at 2 weeks. Morphometric analysis was performed on representative transverse sections and intima medial ratios calculated using computer-aided planimetry. Pro-fibrotic gene expression was assessed with competitive RT-PCR at 2 weeks for metalloproteinase-2, metalloproteinase-9, TIMP-1, collagen III and TGF-β. Sections were stained with picro-sirius red and extracellular matrix deposition was quantified using an image intensifier.

Results: Sirolimus in combination with tacrolimus was associated with the greatest reduction in intimal thickening, median intima medial ratio 0.68 (range 0.36–0.94) compared to treatment with sirolimus and both cyclosporin and tacrolimus, 1.12 (range 1.06–1.68, $P < 0.032$) and untreated controls, 1.47 (1.02–2.04, $P < 0.005$). Furthermore, treatment with sirolimus and tacrolimus significantly attenuated extracellular matrix deposition compared to sirolimus and cyclosporin ($P < 0.023$).

Conclusion: The synergistic benefits of sirolimus in combination with tacrolimus are preferential over those observed with cyclosporin. Randomized trials are warranted to assess if tacrolimus should be the primary calcineurin-inhibitor of choice when used in combination with sirolimus.

Vascular 07

Atheroprotective role of shear forces in superficial femoral artery (SFA) occlusion

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Aim: The course of peripheral arterial occlusive disease in patients with isolated SFA occlusion is benign. In light of the atheroprotective role of high shear-stress, we hypothesized that the haemodynamic and shear-stress alterations in distal arteries subsequent to SFA occlusion may confer atheroprotection, we determined the mean-shear-stress in the popliteal artery of stable claudicants with SFA occlusion in comparison with normal controls matched for age and sex.

Methods: We measured popliteal artery diameter, mean velocity [MV] and flow [MQ] 2 cm distal to medial femoral condyle, on recumbency, using real-time gated Doppler/duplex in 50 limbs with SFA occlusion and 37 normal control limbs. Blood viscosity was calculated from plasma viscosity and haematocrit. Mean-shear-rate $= 4\text{MQ}/\pi\ (\text{Diameter}/2)^3$, mean-shear-stress [dynes cm^{-2}] $=$ mean-shear-rate·viscosity. Data presented as median (inter-quartiles).

Results:

	Diam (cm)	MV (cm s^{-1})	MQ (mL min^{-1})	Shear-rate	Shear-stress
SFA occluded	0.427	13.2	103	244	27
	(0.36–0.487)	(9–15.5)	(73–148)	(153–300)	(25–32)
Control	0.514	8.4	111	127	15
	(0.45–0.6)	(6–10)	(74–138)	(100–182)	(11–20)
P-value	$P < 0.001$	$P < 0.001$	NS	$P < 0.01$	$P < 0.01$

Arteriopaths' plasma viscosity [1.83(1.78–1.94)] mPa s^{-1} was similar to control [1.78(1.7–1.97)] mPa s^{-1}.

Conclusions: Popliteal artery mean-shear-stress in SFA occlusion is significantly higher than in normals, mainly due to higher MV and smaller diameter. Our data suggests that enhancement in haemodynamic shear forces distal to SFA occlusion may act atheroprotectively contributing to the benign vascular outcome in this pattern of disease.

Vascular 08

Regional variation of microvascular abnormalities in individuals at high risk of developing atherosclerotic arterial disease

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Background: Abnormal microvascular reflexes precede the development of peripheral arterial disease; however, regional variation and the role of such abnormalities in dictating the distribution of arterial disease are less clear. We aimed to compare the microvascular response to thermal stimulation in the upper limb with the lower limb in individuals at risk of developing atherosclerotic arterial disease.

Method: Sixteen subjects with no evidence of peripheral arterial disease were recruited for this study and assigned into either a high-risk group (HR) or a low-risk group (LR). Laser Doppler flux was measured over the forearm and the forefoot of all subjects before and after heating the probes to 44 °C.

Results: The mean increase in laser Doppler flux response to thermal stimulation was 574 per cent in the forefoot and 1564 per cent in the forearm in HR group ($P = 0.008$, Wilcoxon) compared with 1332 per cent and 1301 per cent, respectively, in LR group. Furthermore, the difference in the response to thermal stimulation between the two groups in the forefoot was significant ($P = 0.001$, Man–Whitney) while the forearm responses were not.

Conclusion: Microvascular response to thermal stimulation was significantly diminished in the lower limbs of subjects at high risk of developing atherosclerotic arterial disease compared with their upper limbs and with low-risk subjects. Regional variation in these abnormalities may play an important role in the predisposition to and distribution of arterial disease.

Vascular 09

The influence of peripheral vascular disease on the carotid and femoral viscoelastic properties and intima-media thickness in subjects with abdominal aortic aneurysm

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Background: Aortic and carotid stiffness are elevated in patients with abdominal aortic aneurysm (AAA). Failure of aortic compliance to increase with size may account for the higher risk of rupture. Peripheral vascular disease (PVD) frequently coexists with AAA and may further impair the arterial wall mechanics. We studied carotid and femoral viscoelastic properties and intima-media thickness (IMT) in this group of patients at higher mortality risk.

Methods: The viscoelastic properties and IMT of the common carotid and common femoral arteries were determined in 30 AAA patients (15 with PVD) using a duplex ultrasound scanner coupled with wall tracking system. Physiological and biochemical variables were also assessed in these patients

Results: Patients with both AAA and PVD have significantly stiffer carotid and femoral arteries without statistical difference in the IMT. They were matched for age, body mass index, systolic and diastolic pressures, heart rate, presumed cardiovascular load and plasma creatinine, glucose and lipid concentrations.

	PVD & AAA	AAA only	Difference in mean (95% CI)	*P*-value
Viscoelastic index				
CCA, C (% $mmHg^{-1} \times 10^{-2}$)	5.26 (2.00)	8.75 (2.82)	−3.49 (−5.33 to −1.66)	0.001
CCA, E_p (mmHg)	2207 (905)	1268 (432)	938 (408 to 1469)	0.001
CCA, β	22.7 (9.63)	12.6 (4.24)	10.1 (4.6 to 15.7)	0.001
CFA, C (% $mmHg^{-1} \times 10^{-2}$)	4.09 (3.50)	4.64 (2.18)	−0.55 (−2.74 to 1.63)	0.61
CFA, E_p (mmHg)	4906 (4057)	2599 (1169)	2307 (74 to 4540)	0.043
CFA, β	49.0 (40.0)	26.1 (13.2)	23.0 (0.65 to 45.3)	0.044
Intima-media thickness				
CCA (mm)	0.80 (0.25)	0.69 (0.25)	0.11 (−0.07 to 0.30)	0.22
CFA (mm)	1.07 (0.33)	0.89 (0.42)	0.19 (−0.10 to 0.47)	0.19

Conclusion: Reduced carotid and femoral compliance in subjects with PVD and AAA may indicate not only greater cardiovascular risk but also increased risk of aortic expansion and rupture. This may account for the highest mortality seen in this group of subjects in the UK small aneurysm trial.

Vascular 10

In vivo geometric features of femoral bypass distal anastomoses

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Regional Vascular Unit, St Mary's Hospital, London, and Departments of Bioengineering and Aeronautics, Imperial College, London, UK

Aims: Abnormal haemodynamic conditions are implicated in the development of anastomotic myointimal hyperplasia (MIH). We have investigated *in vivo* inter-patient variation in the geometry of distal graft anastomoses, which may strongly influence local flow conditions, MIH formation and clinical outcome.

Methods: The distal anastomotic geometry was acquired *in vivo* by 2-D or 3-D time of flight MR angiography from 10 patients undergoing infrageniculate autologous venous bypass at a median 2 weeks postoperatively. Four patients underwent repeat examinations 13–49 days later. Computer generated 3-D lumen surface reconstructions of the anastomosis were produced and lumen centrelines were extracted. Geometric features including angles between graft and proximal host (α), graft and distal host (β), and proximal and distal host (γ) were calculated. Diameter ratios between the graft and host vessels were calculated from the cross-sectional areas of the three vessels at the reconstructed anastomosis boundaries. A preliminary distal anastomotic planarity index (API) is introduced (for planar anastomoses API = 0) and was evaluated for the patient population studied.

Results:

	Min	Max	Mean	SD
Angles (degrees)				
α	28	101	41.6	20.3
β	144	177	163.8	9.9
γ	113	175	148.4	16.3
Diameter ratios				
Proximal host/graft	0.35	0.79	0.56	0.16
Distal host/graft	0.41	0.96	0.59	0.16
Anastomotic planarity index (API)	0.04	1.4	0.37	0.34

Good agreement was found between the distal anastomotic angles in the initial and follow-up examinations. The differences in the angles varied between 1 and 10 degrees with a mean of 4.3 degrees.

Conclusions: In the early postoperative period distal graft anastomoses exhibit wide variations in angles α, β, and γ, diameter ratios, and planarity. The reported relationship between local haemodynamics and MIH suggests that these geometric features may play a role in MIH development.

Vascular 11

Breaking symmetry: the influence of non-planar configuration on distal anastomotic flow patterns in infra-inguinal bypass grafts

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Objectives: Evidence suggests an inverse relationship between wall shear stress (WSS) and distal anastomotic myointimal hyperplasia (MIH), the commonest cause of bypass graft occlusion. Anastomotic configuration influences flow patterns and WSS, impacting upon anastomotic techniques. The aim of this study was to analyse the influence of non-planar distal anastomoses.

Methods: Contrast enhanced CT scans of distal anastomoses, with three dimensional reconstruction, enabled subjective analysis of *in vivo* non-planar configuration. *In vitro* flow visualization studies and laser Doppler anemometry (LDA) were performed using anastomotic models reflecting these configurations, perfused with glycerol blood analogue under physiological conditions.

Results: The *in vivo* angle between graft and recipient artery ranged from 0 to 45 degrees. *In vitro* flow patterns were altered by an angle of 30 degrees or more with the characteristic vortical flow pattern of a Miller cuff deviated away from the mid-line. In the proximal and distal outflow segments a helical flow pattern occurred, extending several diameters distance down the vessel. LDA velocity vector mapping corroborated the presence of helical flow within the run-off vessels, in direct contrast to symmetrical flow patterns observed within planar models. Out-of-plane configuration within the 5 cm of graft immediately proximal to the distal anastomosis was critical, with no observed influence on flow patterns imparted by more proximal deviation.

Conclusion: *In vivo* non-planar configuration depends in part upon the tunnelling of the graft, and can therefore be influenced. Non-planar anastomotic configurations >30 degrees induce helical outflow patterns which may beneficially increase WSS, thereby potentially inhibiting MIH.

Vascular 12

Vascular endothelial growth factor (VEGF) expression in chronic critical limb ischaemia: a human study

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Although the angiogenic cytokine vascular endothelial growth factor (VEGF) has been used to stimulate angiogenesis in human ischaemic limbs, the endogenous expression of VEGF in patients with critical limb ischaemia (CLI) is unknown Skin and muscle biopsies were obtained from the proximal amputation site, the foot and areas adjacent to gangrene/ulceration in 21 patients undergoing leg amputations for CLI As controls, skin and muscle were obtained from individuals undergoing amputation for congenital deformity ($n = 3$), and skin was collected from individuals during breast reduction surgery ($n = 5$) Total RNA was extracted and VEGF gene expression was detected by RT-PCR. In CLI patients, VEGF was detected in all nine skin samples taken from areas adjacent to the ulcer/gangrene site, and most skin and muscle samples obtained from the thigh (skin, 4/6, muscle 6/6), calf (skin, 12/16, muscle 12/15) and foot (skin, 15/20; muscle 8/11) VEGF was detectable in all six muscle samples from the calf and foot of patients with congenital leg deformity, but in only 1/6 skin samples It was not detectable in breast skin (0/5). The proportion of skin samples in which VEGF gene expression was detectable was higher in patients with CLI (Fishers Exact $P = 0.0002$, CLI *versus* controls) The observation that VEGF expression is detectable in patients with CLI, suggests that any clinical effects that VEGF gene therapy might have may not be mediated via a reversal of deficient VEGF production The clinical significance of the low-level VEGF expression induced by gene therapy transfection approaches requires further consideration

ACPGBI: Six of the Best, Colorectal 17–22 Thursday, 23 May 15.30–17.00

Six of the Best, Colorectal 17

Functional outcome and quality of life after repeat ileal pouch-anal anastomosis (IPAA) for septic and functional complications of ileo-anal surgery

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Aims: Although septic and functional complications may cause IPAA failure, IPAA disconnection with repeat IPAA (R-IPAA) successfully manages these conditions. Experience with R-IPAA is reported, including functional results and quality of life.

Methods: One hundred patients had laparotomy with ileo-anal disconnection and R-IPAA, 80 referred from other institutions. Indications included: chronic anastomotic leak ($n = 27$), pouch-vaginal fistula ($n = 47$), anastomotic stricture ($n = 22$), dysfunctional S-pouch exit conduit ($n = 36$), and previous IPAA excision ($n = 6$). Four patients had Crohn's disease diagnosed before R-IPAA, while 15 Crohn's cases were diagnosed postoperatively.

Results: Twenty-seven new pouches were fashioned, and 73 pouches were reused. Ninety-seven had a temporary ileostomy. Seventy-nine had ileostomy closure to date, and three had no ileostomy. Eighty-two per cent of these patients had a functioning pouch, 32.3 months postoperatively. Two patients were re-diverted and 13 had their pouch excised. The 5-year pouch survival rate was 74 per cent (Kaplan–Meier), better for ulcerative than Crohn's colitis (79 per cent *versus* 53 per cent, $P = 0.06$). There were no differences between septic or nonseptic indications, or between using a new or repaired pouch. Patients had a mean of 8.4 ± 3.9 stools day^{-1}. Forty-two per cent were never/rarely incontinent. Faecal seepage occurred in 50 per cent during the day, and 69 per cent at night. Quality of life was 8.2 ± 1.6, quality of health was 8.0 ± 2.0, and happiness with surgery was 9.0 ± 2.0 (out of 10). A total of 97.3 per cent would undergo R-IPAA again, and 98.6 per cent would recommend it to the others.

Conclusions: R-IPAA is a valid alternative for patients with IPAA failure, including controlled septic conditions. Although pouch failure occurs more frequently with R-IPAA, patient satisfaction is high.

Six of the Best, Colorectal 18

Interventional MRI-guided surgery for anal fistulae

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Aims: To determine whether MRI-guided anorectal surgery was possible and to develop the necessary imaging and surgical techniques to perform image-guided anal fistula surgery.

Materials and methods: A total of 34 patients, 7 with pilonidal sinus and 27 with suspected anal fistulae were recruited into the study. Pilonidal sinus surgery was done as a preliminary procedure prior to performing the more technically demanding fistula procedures. All the procedures were performed in the Signa SPIO 0.5T interventional MRI. Intraoperative imaging, primarily STIR sequences, was used to identify the anatomy and extent of all fistula tracts and septic foci and to ensure the adequacy of the procedure.

Results: In all patients with pilonidal sinus surgery, intraoperative imaging demonstrated the inflammatory changes associated with the lesions and the adequacy of the operation. Imaging failed to demonstrate a fistula in two patients, confirmed by examination under anaesthesia. In the 25 patients in whom a fistula tract or abscess cavity was demonstrated, a postprocedural MRI was done to assess the adequacy of the procedure. Three patients required further surgery, as the intraoperative MRI images demonstrated an incomplete procedure, not obvious on inspection of the surgical field.

Conclusion: MRI-guided surgery for anal fistulae is a feasible option, as it demonstrates the exact anatomy of the primary and secondary tracts and abscesses during the procedure and confirms that all pathology has been adequately addressed surgically. In the future, it may be particularly useful for recurrent and complex anal fistulae.

Six of the Best, Colorectal 19

Fistula-in-ano surgery is not a realistic index procedure for training

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Aims: The ACGBI have identified four index procedures for training and suggests the minimum numbers to achieve competency. For fistula-in-ano (FIA) surgery this number is 30. No recommendation of what proportion should be 'complex' FIA has been made. The central unit in our region has a regional referral practice for complex FIA. This provides an ideal opportunity to assess exposure of HSTs to complex FIA.

Method: We reviewed the notes of all patients referred for FIA surgery over an 18-month period using the unit's prospective audit databse. Fistulas were analysed using Parks' classification.

1. Subcutaneous (simple)
2. Intersphincteric (simple)
3. Intersphincteric + abscess or secondary track above the dentate line (complex)
4. Transsphincteric at the dentate line or above (complex)
5. Transsphincteric + abscess or secondary track (complex)
6. Supra-sphincteric (complex)
7. Extra-sphincteric (complex)

Results: Eighty-five operations for FIA were performed. Twenty-nine of 58 patients with simple FIA (50%) and 13/27 patients with complex FIA (48%) were operated on by higher surgical trainees. An average of 57 FIA operations per year were performed; 39 simple and 18 complex. Trainees performed 19 and 9 of these respectively.

Conclusion: Over a period of 1 year this colorectal unit provided 93% of the minimum number of FIAs for trainees to achieve competency levels as defined by the ACPGBI. However, most were simple with complex fistulas requiring significant consultant input. The competency of colorectal HSTs required to manage 'complex' FIA as independent practitioners remains uncertain without the availability of more detailed guidelines on what constitutes a complex FIA and the degree of exposure to these procedures during training.

Six of the Best, Colorectal 20

Whole-body FDG-pet for surgical staging of colorectal liver metastases

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Aims: Surgical resection is the only potential cure for patients with colorectal liver metastases (CLM), but patient selection relies on accurate preoperative staging. The aim of this study was to assess the accuracy of routinely using whole-body FDG-PET (WBPET) for preoperative staging of patients being considered for resection of CLM.

Methods: A prospective study of patients referred for possible hepatic resection was undertaken. Patients were initially staged by spiral CT and subsequently underwent WBPET. These modalities were considered independently before findings were compared and a decision regarding patient management was made. Accuracy of each modality was compared with histology, clinical/radiological follow-up or operative findings if appropriate.

Results: Twenty-nine patients were recruited. Ten solitary CLM were correctly identified by both WBPET and CT. Nineteen patients had multiple CLM or extrahepatic disease, of these CT correctly staged seven patients (36 per cent), understaged 10 patients (53 per cent) and overstaged two (11 per cent). WBPET correctly staged 18 patients (95 per cent) and overstaged just one (5 per cent). WBPET was more sensitive and specific (100 and 92 per cent, respectively) for detecting multiple CLM and extrahepatic disease compared to CT (41 and 83 per cent). As a result of routine WBPET, patient management was altered in 10 patients (34 per cent), of whom four (14 per cent) avoided inappropriate surgery

Conclusions: WBPET is both more sensitive and specific in the preoperative staging of CLM, and we recommend its inclusion in the management algorithm of all patients being considered for hepatic resection Altered patient management such as avoiding inappropriate laparotomy may also ultimately lead to financial savings.

Six of the Best, Colorectal 21

Intravenous *versus* intrahepatic arterial 5FU/leucovorin for colorectal liver metastases: preliminary results of a randomized trial

I. Taylor on behalf of the MRC and EORTC Colorectal Cancer Groups and all the participants

Department of Surgery, Royal Free and University College Medical School, London, UK

Aims: Intrahepatic arterial (IHA) therapy should deliver higher doses of drug to the liver with reduced overall systemic exposure. An IHA regimen was designed which used the same drugs and schedule, and achieved similar toxicity and steady-state venous 5FU levels as the standard de Gramont IV regimen Patients with advanced colorectal liver metastases were randomized to either IV or IHA.

Methods: In total 290 patients were randomized from 16 centres in 6 years The median age was 62 years, 70 per cent were male, 68 per cent had colon cancer. Of the 145 patients allocated to IV, 14 per cent did not receive any allocated therapy, but 78 per cent received six cycles of chemotherapy Of the 145 IHA patients, 37 per cent did not start and only 35 per cent received six cycles. The additional problems in the IHA group were inability to insert the catheter, or infected, leaking, or blocked catheters. Of the patients who did not receive six cycles of IHA therapy, 52 per cent switched to IV therapy Similar levels of toxicity and quality of life were reported in both arms.

Results: There was no clear evidence of a difference in progression-free survival (HR 0.90, 95 per cent CI· 0.71–1.16; $P = 0.42$) or overall survival (HR 1.03, 95 per cent CI: 0.79–1.33, $P = 0.85$) From randomization median, and estimated 1- and 2-year survival were 14.1 months in both groups, and 60 and 57 per cent, and 26 and 22 per cent for IV and IHA, respectively.

Conclusions: This trial suggests that there is no obvious role for this IHA regimen in the management of hepatic metastatic colorectal cancer

Six of the Best, Colorectal 22

Effectiveness of a rapid-access rectal bleeding clinic in the prevention and treatment of colorectal cancer

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Aims: To audit the effectiveness of a Rectal Bleeding Clinic (RBC) in detecting premalignant colonic lesions and early colorectal cancers.

Methods: All 1175 consecutive patients referred to the RBC from November 1997 to September 2001 were assessed by detailed history, clinical examination and flexible sigmoidoscopy Those with neoplastic lesions diagnosed on flexible sigmoidoscopy underwent subsequent colonoscopy The final definitive histology of each patient was confirmed from the histology department database which was also used to identify a control group of consecutive patients with colorectal cancer diagnosed in conventional outpatients' clinics

Results: One hundred and thirty eight patients (11.7 per cent) had significant neoplastic lesions Of these, 88 had adenomatous polyps and 50 patients had invasive cancer Of the invasive cancers, 18 (36 per cent) patients had Duke's A lesions, as compared to 16 per cent of the control group of patients coming through conventional clinics during this period Sixteen patients (8.1 per cent) aged 40–49 years were diagnosed as having neoplastic lesions (10 with low-grade dysplasia, one with high-grade dysplasia and five with invasive cancer)

Conclusions: A rapid-access RBC enables detection of a higher proportion of potentially curable early colorectal cancers than conventional clinics in addition to a large number of premalignant lesions which can be treated endoscopically with subsequent colonoscopic surveillance Our findings in the 40–49 years age group lead us to question the wisdom of a minimum age for these clinics of 50 years as practiced in some centres

ASGBI: Six of the Best, General 19–24

Thursday, 23 May 16.00–17.00

Six of the Best, General 19

Reduction in junior doctors' hours: implications for surgical training

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Aims: Hours of work of junior doctors have been progressively cut over the last few years, while the period of surgical training has remained fixed and as a result there are concerns that surgical trainees are not receiving adequate training. We studied the impact of changing on-call rota to a partial shift rota on the operative experience of junior surgeons in a district general hospital.

Methods: We retrospectively looked at operative experience (excluding endoscopies and minor procedures) gained over 8 months. For the first 4 months, junior staff consisted of four specialist registrars (SPR) and one senior house officer (SHO) working a one in five on-call rota contracted for 72 h week^{-1}. In the following 4 months, junior staff consisted of four specialist registrars and two SHOs (one additional SHO covering nights and weekends) working a partial shift rota contracted for 64 h week^{-1}. The total number of operations performed (elective and emergency procedures) were recorded by analysis of trainees log books.

Results: The results are summarized below. Specialist registrars' total operative experience declined by 10 per cent; elective procedures declined by 11 per cent and emergency procedures declined by 5 per cent. No significant differences were seen in difficulty of operation or level of supervision. SHOs experience increased, but not as much as expected given that two were employed compared to one in the first period.

		SPRs		SHOs	
	Total	Elective	Emergency	Elective	Emergency
Period 1	712	498	138	58	18
Period 2	687	443	131	81	32
Change		−55 (11%)	−7 (5%)	+23 (40%)	+14 (77%)
Total change		−62 (10%)		+37 (49%)	

Conclusion: The reduction in hours has made a significant impact on operative training exposure for specialist registrars.

Six of the Best, General 20

A comparison of operative experience for basic surgical trainees in 1992 and 2000

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Aims: To compare the operative activity of two first-year basic surgical trainees working in comparable district general hospitals in 1992 and 2000.

Methods: Prospectively collected operating log-book data was compared for the first 15 months of general surgical training of a BST in 1992 to that of a similar trainee in 2000. Both elective and emergency cases were analysed. The surgical speciality and the trainees' role in the case were recorded. Several index operations were also compared.

Results: Emergency case numbers were similar (145 in 1992 and 133 in 2000), however, the elective workload was almost halved (435 in 1992 and 225 in 2000). The trainee in 1992 assisted, was supervised and performed more operations, in all grades of procedure, than the trainee in 2000 (Table 1), as a result the trainee in 2000 spent the majority of his time assisting cases but the trainee in 1992 had moved on to be the principal operator.

Table 1

	Assisted	Supervised	Performed
1992	221	138	76
2000	153	68	4

The trainee in 1992 had a greater exposure to most surgical subspecialties than the trainee in 2000 (Table 2).

Table 2

	General	Upper GI	Lower GI	Vascular	Breast
1992	171	66	74	91	33
2000	79	48	21	15	62

In all index procedures (appendicectomy, varicose veins, hernia and upper GI endoscopy), the 1992 trainee did appreciably more.

Conclusions: The emergency workload was similar, however, the elective workload was reduced by 48 per cent, resulting in a reduction in the training available to the 2000 trainee. Training in 1992 was more 'general' compared to 2000.

Six of the Best, General 21

Emergency surgeon-performed hepatobiliary ultrasound

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Department of Surgery, Cork University Hospital, University College Cork, Cork, Ireland

Aims: Acute hepatobiliary pathology is a common general surgical emergency, and diagnosis requires imaging of the biliary tree by ultrasound. Herein, we examine the accuracy and impact of surgeon-performed ultrasound (SUS) on the diagnosis of emergent hepatobiliary pathology.

Methods: This prospective study, over a 6-month period, enrolled all patients with symptoms or signs of acute hepatobiliary pathology. Patients provided informed consent and underwent both SUS and standard radiology-performed ultrasound (RUS). SUS was performed using a 2–5 MHz broadband portable ultrasound probe by two surgeons trained in ultrasound and RUS using a 2–5 MHz fixed unit. SUS results were correlated with RUS and pathological diagnoses.

Results: Fifty-three consecutive patients received a total of 106 ultrasound investigations. SUS agreed with RUS in 50 of the 53 patients (94.3 per cent). SUS accurately detected cholilithiasis in all but two cases and no patients were inaccurately diagnosed to have cholilithiasis at SUS (95 per cent sensitivity and 100 per cent specificity). As an overall complimentary diagnostic tool, SUS provided the correct diagnosis in 94 per cent of the patients. Time to scan was significantly shorter following SUS (3.07 h *versus* 12 h, $P < 0.05$).

Conclusions: SUS offers excellent alacrity in the diagnosis of emergency hepatobiliary pathology. SUS may be a useful adjuvant in the emergency management of hepatobiliary disease.

Six of the Best, General 22

Complex liver trauma: a 10-year experience

R.D. Brammer, S.R. Bramhall, D.F. Mirza, A.D. Mayer, D. Candinas, P. McMaster and J.A.C. Buckels

The Liver Unit, Queen Elizabeth Hospital, Birmingham, UK

Aims: To show that most liver trauma can be managed conservatively and that early abdominal CT reduces the frequency of operative treatment in nonspecialized centres which carries a high mortality.

Methods: We retrospectively analysed data from 52 consecutive patients referred to the Queen Elizabeth Hospital, Birmingham over a 10-year period to identify best practice in the management of these injuries.

Results: The majority of injuries occurred as a result of road traffic accidents. Seventy-five per cent of the patients were stable at presentation to the referring hospital. In 70 per cent of the patients, the liver injury was a component of multiple trauma. USS, CT and no radiological investigation were used in the referring hospital in 35, 48 and 17 per cent, respectively. Operative management was undertaken in the referring hospital in 50 per cent of patients. The overall mortality in the series was 21 per cent and increased with increasing grade of severity. The mortality in patients managed operatively at the referring hospital was 31 per cent and in those managed conservatively was 15 per cent ($P = \text{ns}$). The median time from arrival to surgery in the referring hospital was 4 h in haemodynamically stable patients and 3 h in haemodynamically unstable patients.

Conclusion: Our data suggests that the majority of cases of liver trauma may be managed conservatively and that operative management in nonspecialized units carries high mortality and morbidity. Abdominal injuries should raise a high index of suspicion of liver injury, and abdominal CT should precede laparotomy (even in some haemodynamically unstable patients) to facilitate early discussion with a specialist centre.

Six of the Best, General 23

Magnetic resonance-guided surgery for resection of soft-tissue tumours

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Aim: Evaluate role of intraoperative image guidance in soft-tissue sarcoma surgery. We describe a method of determining adequate resection margins with intraoperative MRI in seven patients.

Patients and methods: This preliminary report describes seven patients with soft-tissue sarcomas who underwent MR-guided excision of their lesions. MR excision was performed using titanium instruments and an ultrasonically activated scalpel. After resection of the lesion, further MRI sequences confirmed complete excision with clear margins. The skin was closed primarily in each case without the need for any plastic reconstruction.

Results: All seven procedures were undertaken without anaesthetic or surgical complication. Pre-procedure MR in all cases demonstrated that the lesions were considerably more extensive than was apparent from clinical examination. Immediate postexcision imaging in all cases confirmed complete excision. The rapidity of the scanning preceded the development of local oedema at the surgical site, which would also have produced high signal. Histological examination confirmed clear surgical margins.

Conclusions: MR-guided surgery may reduce the incidence of local recurrences and specimen margins positive following resection and ensures complete removal of all malignant tissue at a single procedure, which can be tailored to the tumour extension thus reducing the amount of noninvolved tissue removal.

Six of the Best, General 24

Conscious pain mapping using laparoscopy under local anaesthetic and sedation

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Aims: Patients in whom extensive investigations have failed to identify the cause of abdominal pain present a challenge to surgeons. We present our initial experience of using laparoscopy under local anaesthetic and sedation in the diagnosis of chronic abdominal pain.

Methods: Nine patients with chronic abdominal pain and multiple normal investigations underwent laparoscopy under local anaesthetic and sedation. By touching and grasping intra-abdominal viscera and peritoneum, an attempt was made to reproduce the patient's pain.

Results: Two patients were found to have pain arising from the gall bladder and subsequently underwent laparoscopic cholecystectomy with resolution of their symptoms. A third patient had a clinical presentation of chronic acalculous cholecystitis and a normal laparoscopy. She decided to undergo laparoscopic cholecystectomy, which cured her pain. Another patient had pain arising from the appendix, which resolved after an appendicectomy. Another patient had pelvic adhesions and after adhesiolysis is pain free. One patient declined surgery for adhesions, which produced chronic pain and another patient had adhesions secondary to an appendicectomy but did not cause any pain at the time of her laparoscopy. Another patient awaits a repeat laparoscopic adhesiolysis. One patient had a normal laparoscopy and a gastroenterologist has made a diagnosis of irritable bowel syndrome.

Conclusion: Laparoscopy can be carried out in the awake patient, who is then usually able to differentiate the type of pain experienced during conscious pain mapping. Long-term effectiveness and diagnostic accuracy has not yet been established.

ASGBI: Upper GI 19–27

Thursday, 23 May 16.00–17.30

Upper GI 19

Major liver resection in the elderly

N. Ahmad, K.V. Menon, S.W. Fenwick, K.V. Ravindra, N. Pearce, K.R. Prasad, G.J. Toogood and J.P.A. Lodge

The Hepatobiliary Unit, St James's University Hospital, Leeds, UK

Aims: The safety of major liver resection is improving with advances in surgical techniques and anaesthesia. We assessed the morbidity and mortality with major liver resection in patients over the age of 70 years.

Methods: Between 1993 and 2001, over 500 patients underwent major liver resections. Of these, 55 (resection of three or more segments) were performed in the elderly. ASA grade, intraoperative blood transfusions, length of intensive care (ITU) and hospital stay, morbidity and in-hospital mortality were recorded prospectively.

Results: Fifty-five patients (median age 73 (70–85) years; M:F = 23:22) underwent a major liver resection, the vast majority for colorectal liver metastases (colorectal: 42; hepatocellular carcinoma: 8; cholangiocarcinoma: 1; benign: 4). The median ASA grade was 2 (1–3). Only a third of patients had blood transfusion, median 3 (1–11) units. There were five in-hospital deaths (9 per cent) – four deaths were in the early period ($n = 20$, 1993–1997) and only one death in the latter period ($n = 35$, 1998–2001). Major morbidity was seen in 20 patients (36 per cent) (cardiac: 5; respiratory: 5; gastrointestinal bleed: 3; transient liver failure: 3; cerebrovascular accident: 1; intra-abdominal collection: 1; small bowel fistula and obstruction: 1). Only five patients needed ITU. The median hospital stay was 11 (5–139) days.

Conclusion: Major hepatic resection can be safely performed in the elderly with low morbidity and mortality. However, these patients need careful cardiac and respiratory assessment, as these are the major causes for morbidity in the postoperative period.

Upper GI 20

Extending right-sided liver resection – how far can we go?

K.V. Menon, N. Ahmad, K.R. Prasad, G.J. Toogood and J.P.A. Lodge

The Hepatobiliary Unit, St James's University Hospital, Leeds, UK

Aims: In hepatic right trisectionectomy (previously, trisegmentectomy), it is sometimes necessary to extend the resection to the left of falciform ligament to achieve tumour clearance. We have assessed the outcomes following such extended resections.

Methods: Between 1993 and 2001, 112 patients underwent right trisectionectomy for malignancy. In 37 of these patients, resection extended to the left of falciform ligament (30: colorectal; 4: sarcoma; 2: neuroendocrine; 1: oesophageal). These resections were either contiguity or as a segment 2/3 metastasectomy. (M2, M3 or M2 + 3). In contiguity (IC) resections of either segment 2 (IC2) or 3 (IC3) or both (IC2 + 3) were done to achieve tumour clearance. Postoperatively, liver function was monitored for at least 1 week.

Results: Of the 37 patients who underwent a right trisectionectomy extending beyond the falciform ligament, 19 had an IC resection (IC2 = 5, IC3 = 8, IC2 + 3 = 4). Seven patients had an IC resection and a metastasectomy (IC + M2 = 1, IC + M3 = 3, IC + M2 + 3 = 3). Thirteen patients had a segment 2 or 3 metastasectomy alone [median 2 (1–5) metastases]. The median hospital stay was 10 (6–23) days. There was one (2.7 per cent) in-hospital death. Three patients developed bile leak and two had postoperative haemorrhage that needed re-exploration. Thirteen patients (35 per cent) had transient hepatic dysfunction. Five patients died from recurrent disease and two from other causes. Twenty-nine patients are currently alive, five with recurrent disease.

Conclusion: Right-sided liver resection can be extended across the falciform ligament safely, enabling clearance of the tumour with acceptable mortality.

Upper GI 21

Inoperable hepatocellular carcinoma treated with transcatheter arterial chemoembolisation (TACE): an analysis of prognostic factors in 5-year survivors

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Centre for the Study of Liver Diseases, Departments of Surgery and Radiology, University of Hong Kong Medical Centre, Queen Mary Hospital, Hong Kong, China

Aim: To evaluate the long-term survival benefit of TACE in patients with inoperable hepatocellular carcinoma and determine prognostic factors by analysis of actual 5-year survivors.

Methods: Pretreatment variables were analysed from a prospective database of 335 consecutive patients from January 1989 to December 1996. Univariate and multivariate analyses were performed to identify factors predictive of 5-year survival.

Results: Complete 5-year follow-up (median 91 months) was obtained on 320 patients (275 males) who underwent a mean of 3 TACEs (range 1–41) for inoperable HCC. Median age was 59 years (range 19–83). The majority were Child-Pugh grade A (81 per cent). Median tumour size was 9 cm (1–28 cm). There were 98 (30.6 per cent) 1-year and 36 (11.3 per cent) 3-year survivors. Among the 25 (7.8 per cent) 5-year survivors, survival ranged from 60.7 to 138.8 months (median 72.3 months) and tumour size from 1 to 21 cm (median 5.3 cm), with eight tumours greater than 10 cm. On univariate analysis, female gender ($P = 0.037$), absence of ascites ($P = 0.028$), platelet count $< 150 \times 10^{9}\,\mathrm{L}^{-1}$ ($P = 0.011$), albumin $> 35\,\mathrm{g\,L}^{-1}$ ($P = 0.04$), α-fetoprotein $< 1000\,\mathrm{ng\,mL}^{-1}$ ($P = 0.007$), absence of venous invasion ($P = 0.011$), unilobar distribution ($P = 0.027$), less than three tumours ($P = 0.015$), and tumour size < 8 cm ($P = 0.021$) were significant predictors of 5-year survival. Albumin $> 35\,\mathrm{g\,L}^{-1}$ ($P = 0.014$), unilobar distribution ($P = 0.011$) and α-fetoprotein $< 1000\,\mathrm{ng\,mL}^{-1}$ (0.014) were independent predictors of 5-year survival on multivariate regressional analysis.

Conclusion: Five-year survival is possible in patients with inoperable hepatocellular carcinoma. Poor prognosis is associated with a bilobar distribution, an α-fetoprotein level of $> 1000\,\mathrm{ng\,L}^{-1}$ on presentation and poor underlying liver function.

Upper GI 22

Routine magnetic resonance cholangiopancreatography and intraoperative cholangiogram in the evaluation of common bile duct stones

S.J. Dalton, S. Balupuri and J. Guest

Department of Surgery, Wansbeck Hospital, Newcastle upon Tyne, UK

Background: Magnetic resonance cholangiopancreatography (MRCP) is a safe and sensitive investigation for the imaging of common bile duct pathology. When used to exclude common bile duct (CBD) stones MRCP may obviate the need for intraoperative cholangiogram (IOC). In this prospective study, we looked at the single centre results of patients who underwent cholecystectomy with IOC following preoperative MRCP.

Methods: Over a period of 1 year, 69 patients (24 male and 45 female), mean age 59 (range 19–86) years were investigated by MRCP prior to cholecystectomy. All patients underwent IOC. Inclusion criteria for MRCP consisted of derangement of liver function tests and/or history of jaundice in cases of US proven cholelithiasis.

Results: Sixteen ($n = 69$) had suspected stones or filling defects on MRCP, all but two of these were confirmed to be stones on IOC. In only one patient was a stone visualized on IOC and not seen on MRCP.

Conclusions: MRCP may be the only preoperative investigation needed for exclusion of CBD stones, obviating the necessity for intraoperative cholangiogram.

Upper GI 23

Pancreatico-biliary reflux and cystic duct morphology: relation to common bile duct stones and endoscopic sphincterotomy

S Tranter, M Thompson, M Gillett and L Biddlestone

Department of Surgery, Department of Biochemistry and Department of Histopathology, Southmead Hospital, Bristol, UK

Aims: Endoscopic sphincterotomy (ES) may result in bile duct malignancy in the long term· reflux of pancreatic enzymes into the bile duct may be contributory To test this hypothesis, we assessed the degree of pancreatico-biliary reflux and associated biliary mucosal inflammation in patients with and without bile duct stones (CBDS) and after sphincterotomy

Methods: Bile was aspirated via cystic duct cannulation in 65 patients undergoing laparoscopic cholecystectomy ± ductal exploration. The quantity of chymotrypsin, lipase and amylase was measured in each sample Comparisons were made between the following six patient groups: those with no current or previous CBDS ($n = 14$), patients without pancreatitis who had passed CBDS ($n = 7$), patients with pancreatitis with previous CBDS ($n = 7$), patients with current CBDS ($n = 23$), and patients who had undergone a previous ES, with ($n = 7$) and without current CBDS ($n = 7$). The degree of cystic duct inflammation was assessed for each patient by a histopathologist blinded to the clinical history.

Results: Patients with no previous CBDS and those without pancreatitis with previous CBDS had negligible biliary concentrations of pancreatic enzymes and no cystic duct mucosal inflammation Patients with current CBDS had mildly elevated amylase/lipase concentrations (4.1/2.2 activity units mL^{-1}) and negligible cystic duct inflammation In contrast, patients who had undergone ES (±CBDS) and those with recent pancreatitis had a moderate degree of cystic duct inflammation and higher amylase/lipase concentrations (24/16.2 activity units mL^{-1} $P < 0.05$ after ES, 136/61.8 activity units mL^{-1} $P < 0.001$ after pancreatitis). Chymotrypsin was only detectable in patients who had pancreatitis (6 activity units mL^{-1})

Conclusions: High concentrations of pancreatic enzymes are only found in bile after a recent attack of acute pancreatitis and subsequent to ES. in both cases, there is associated mucosal inflammation. These changes may contribute to the late sequelae of ES

Upper GI 24

Minimally invasive thoracoscopic splanchnicectomy (MITS) for pain secondary to chronic pancreatitis: a prospective audit

C B Ó Súilleabháin, N Menezes, C.J McKay, C R. Carter and C W Imrie

Lister Department of Surgery, Glasgow Royal Infirmary, Glasgow, Scotland, UK

Aim: To prospectively evaluate the short- and medium-term response to bilateral thoracoscopic splanchnicectomy in a consecutive series of patients with pain secondary to chronic pancreatitis (CP)

Methods: All patients completed a 10-point visual analogue pain scale (VAS) and a detailed analysis of analgesic requirements was made before surgery, 1 day after surgery and at 6, 12, 24 months and yearly thereafter.

Results: In 44 (26 males) of 68 patients undergoing MITS, the indication was CP (alcohol 73 per cent, idiopathic: 20 per cent, familial 7 per cent). The median age was 43.5 years (23–68 years) Twelve patients (27 per cent) had undergone previous pancreatic resection, 17 (39 per cent) a pancreatic drainage procedure and 2 (4 per cent) a coeliac axis block Bilateral splanchnicectomy was completed in 42 patients (95 per cent). Five patients had intrathoracic bleeding, one required a thoracotomy, the remainder were managed successfully thoracoscopically Follow-up was available on 42 patients Median follow-up was 2 years (6–60 months) Median hospital stay was 2 days (1–19 days) Ninety-six per cent of patients required regular daily opioids prior to splanchnicectomy The median preoperative VAS was 9 ± 0.16 and was significantly reduced on the first postoperative day (VAS 2 ± 0.45, $P < 0.0001$). At 6, 12 and 24 months, the median VAS remained significantly lower ($P < 0.0001$, respectively) However, patients requiring daily opioid analgesics rose from 22 per cent at 6 months to 39 per cent at 1 year and 54 per cent at 2 years.

Conclusion: MITS offers substantial short-term relief from pain in patients with chronic pancreatitis The longer term benefits do not appear as good, although the cycle of opioid dependency is often broken.

Upper GI 25

Laparoscopic ultrasound is capable of replacing operative cholangiography

S. Tranter and M Thompson

Department of Surgery, Southmead Hospital, Bristol, UK

Aims: Intraoperative cholangiography (IOC) is time consuming, requires radiation and sometimes fails, in contrast to laparoscopic ultrasound (LUS), a comparatively quick, safe and noninvasive technique. All previous studies comparing LUS to IOC were not blinded we have rectified that weakness by blinding the observer.

Methods: LUS and subsequently IOC were performed on 135 patients undergoing laparoscopic cholecystectomy by different operators blinded to the patient details. Biliary anatomy and ductal stone data were collected prospectively A positive finding from either technique resulted in laparoscopic duct exploration recording the presence (in 49 cases) and number/size of the ductal stones (CBDS)

Results: LUS adequately visualized the extra-hepatic biliary tree in 98 per cent of cases compared to 83 per cent for IOC. A total of 19/23 IOC failures related to difficult cystic duct cannulation. LUS correctly identified stones in 48/49 patients with no false positive findings (LUS sensitivity: 98 per cent; specificity 100 per cent, PPV· 100 per cent; NPV: 99 per cent). IOC accurately demonstrated CBDS in 44/49 patients with only one false positive demonstration (IOC sensitivity 90 per cent, specificity 99 per cent, PPV 98 per cent, NPV 94 per cent) Both LUS and IOC underestimated the total number of stones in 21 per cent of patients. LUS accurately determined the stone size in 95 per cent of cases and IOC in 89 per cent of cases.

Conclusions: LUS had a higher success rate of bile duct visualization than IOC, was a more sensitive and specific method of detecting CBDS, and is comparable to IOC in detecting the size and number of the CBDS. LUS is therefore a satisfactory alternative to cholangiography in the detection of ductal stones

Upper GI 26

Clinical and quality-of-life outcomes during tri-modality treatment for oesophageal cancer

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Aims: Neo-adjuvant chemoradiation before surgery for oesophageal cancer is designed to downstage loco-regional disease and increase the chance of cure. Because this intensive tri-modality approach may have major toxicity, increase operative risks and impact on quality of life (QoL), this study examined clinical and QoL outcomes during neo-adjuvant treatment for oesophageal cancer

Methods: Between November 2000 and December 2001, 26 patients with loco-regional (stage IIb/III) oesophageal cancer commenced treatment with infusional chemotherapy and radiotherapy followed by oesophagectomy. QoL was assessed using the QLQ-C30 and QLQ-OES24 before treatment, and at 7 and 12 weeks during neo-adjuvant treatment, before surgery and 6 weeks after surgery

Results: A total of 19 out of 26 completed tri-modality treatment (two postoperative deaths), seven (27 per cent) did not proceed to oesophagectomy (four deaths, two disease progression, one deteriorating general health) Functional aspects of QoL (physical, social and emotional well being) deteriorated during neo-adjuvant treatment, stabilized before surgery then fell again in the early postoperative phase. Most symptom scores worsened during neo-adjuvant treatment except for difficulties with eating, which improved. Six weeks after surgery, all symptom scores were the same or worse than at diagnosis. Significantly better physical function scores ($P = 0.005$) and less problems with dysphagia ($P = 0.009$) were reported by patients who completed three treatment schedules than by patients who did not proceed to surgery.

Conclusions: Tri-modality treatment is associated with significant mortality, a quarter of patients do not undergo oesophagectomy and QoL during the neo-adjuvant and surgical stages deteriorates markedly. These major problems must be offset against any potential long-term survival benefits.

Upper GI 27

Quantification of FDG-PET scans in patients with oesophageal and gastric cancer. A study of 40 patients

G.W. Couper, D. McAteer, F. Wallis, A. Welch, M. Norton and K.G.M. Park

Departments of Surgery, Biomedical Physics and Radiology, University of Aberdeen and Aberdeen Royal Hospitals NHS Trust

Aims: Positron emission tomography is increasingly used in the detection, staging and assessment of response to therapy in patients with upper GI cancer, but no agreement exists on the method of quantification of scans to allow comparison of results. Quantification can be achieved by the calculation of simple ratio measurements, uptake values or more complex influx constants, and we aim to determine which method of quantification is required to obtain useful clinical information.

Methods: A total of 40 patients underwent FDG-PET imaging. All patients underwent static imaging with the calculation of tumour/liver ratios (TLR) and standardized uptake values (SUV) for quantification. Nineteen patients had both static and more complex dynamic imaging, allowing in addition the calculation of K, the influx constant of FDG. A total of 16 patients had PET scans pre- and post-chemotherapy.

Results: FDG-PET detected 39 of 40 lesions, not detecting an area of linitus plastica in one patient. Correlation coefficients were calculated between methods of quantification. A correlation coefficient of 0.92 existed between TLR and SUV. Agreement was poor between TLR and SUV compared with K-values (0.51 and 0.41). In contrast, the correlation coefficient was 0.9 between TLR and K-value quantification when detecting change in activity following chemotherapy. Changes in activity ranged from a 15 per cent increase to a complete response following chemotherapy.

Conclusions: FDG-PET is reliable in the detection of upper GI tumours. It shows great potential in the noninvasive detection of response to chemotherapy, and this can be achieved by a shorter imaging time and less complex calculation of tumour/liver ratios.

ASGBI: Audit 01–09

Friday, 24 May 08.30–10.00

Audit 01

An audit of varicose vein surgery: the patients' perspective

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Department of Vascular Surgery, Mayday University Hospital, Surrey, UK

Aims: To identify patients' reasons for requesting varicose vein surgery and their subsequent satisfaction of surgical outcome in order to identify measures for improvement.

Methods: A questionnaire was sent to 404 patients who underwent surgery for varicose veins over a 2-year period from August 1994 to July 1996 under the care of a single vascular consultant. Information was requested regarding preoperative symptoms and satisfaction with regard to the outcome of surgery.

Results: A total of 335 (83 per cent) patients ($F = 248$, $M = 87$) completed the questionnaire. The symptoms identified were aching (59 per cent) and appearance (54 per cent), itching by 33 per cent, acute pain by 24 per cent, ulcers for 7 per cent and 22 responders reported swelling, phlebitis, eczema, bleeding and restlessness. Eighty-one patients (24 per cent) presented with recurrent varicose veins. There were 69 (21 per cent) patients dissatisfied following surgery. Patients with ulcers preoperatively were satisfied (92 per cent). We found significantly higher dissatisfaction rates among females (24 per cent *versus* 11 per cent $P < 0.025$), those having bilateral surgery, those whose operation was performed by junior staff, those who noticed complications of surgery (29 per cent, $P < 0.005$), those who did not find an improvement in their symptoms postoperatively (75 per cent, $P < 0.0005$) and those who were dissatisfied with the explanation given prior to surgery (61 per cent, $P < 0.0005$).

Conclusions: The majority of patients are satisfied with the result of varicose vein surgery. We have identified modifiable factors that may influence satisfaction. We suggest that supervision of junior staff and provision preoperatively of information on the cosmetic outcome of varicose vein surgery and the expected postoperative course may improve overall satisfaction.

Audit 02

Misleading mortality data in league

K Gill, P. Clarke and C.R R. Corbett

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Aim: Surgeons cannot ignore league tables: in 2001 we received only one star in the Department of Health league table. A contributory factor was an excess of in-hospital deaths and the Trust asked us to look at mortality rates in general surgery.

Methods: Hospital Episode Statistics are derived from patient administration system (PAS) and an outside firm compares our performance against a 'peer group'. We have our own departmental database. Scrutiny of the data presented by the outside firm for the years 1999/2000 and 2000/2001 raised concerns. Specifically, there was a higher than expected death rate 'postprocedure' (48/64 total deaths). The 48 deaths represented 1.54 per cent of all admissions, comparing badly with 1.11 per cent in the peer group ($\chi^2 = 4.59$, $P < 0.05$). This demanded a review of all case notes, computer records and all diagnostic, procedure and operation codes.

Results: Only 26/64 of the reported deaths were truly postoperative. The 22 excess deaths ($48 - 26$) had procedures such as i.v. administration of morphine or antibiotics, transfusion, insertion of CVP line, urethral catheterization, fine needle biopsy of mass and diagnostic arteriography. These deaths were wrongly interpreted as postoperative, despite DoH definitions. Our total death rate (2.05 per cent) and the true postoperative death rate (0.83 per cent) were lower than in the peer group (2.10 and 1.11 per cent), but not significantly different.

Conclusions: We conclude that clinicians must be more closely involved in preparing these data. They are the basis for submissions to NCEPOD, so the error is repeated and the work of our department is denigrated nationally.

Audit 03

The surgical acute care unit: an effective method of reducing intensive care demand with no adverse effect on mortality

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Aims: Surgical acute care units (SACU) are being established for the care of patients who do not meet criteria for admission to intensive care or high dependency units, but require more care than can be easily provided on a normal surgical ward. The aim of this study was to audit the workload, case-mix and mortality during the 6 months of a SACU.

Methods: A retrospective case-note review of all patients admitted to the SACU over a 6-month period was performed. The patients' demographics, admission source, diagnosis, discharge destination and mortality rates were recorded. In addition, POSSUM scores were calculated for each patient and the P-POSSUM equation applied to estimate predicted mortality. Results are expressed as mean (SD, range).

Results: A total of 131 patients were admitted during the 6-month period (43 female, 33 per cent) for a mean duration of 2.9 days. Sixty-three (48.1 per cent) were admitted from the critical care wards and 46 (35.1 per cent) directly from theatre. Those admissions from theatre included 14 patients who would previously have required high dependency care in the initial postoperative period (nine carotid endarterectomies and four subtotal colectomies). The mean POSSUM physiology and operative severity scores were 22.0 (8.9, 13–53) and 17.6 (5.64, 8–32), respectively. Mean predicted mortality was 14.3 per cent (0.7–88.8), whilst actual in-hospital mortality was 6.1 per cent (eight patients).

Conclusion: The Surgical Acute Care Unit reduces the demand on scarce critical care resources with no adverse effect on mortality.

Audit 04

The role of national guidelines in surgical audit: endocrine surgery in southeast Scotland

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Aims: Following publication of 'Guidelines for the Management of Endocrine Disease' by the British Association of Endocrine Surgeons (BAES) in November 2000, this study aims to determine the number of surgeons within southeast Scotland performing endocrine surgery, their awareness of and adherence to guidelines 1 year after publication.

Methods: Questionnaires were circulated in December 2001 to all surgeons performing thyroid surgery, involving questions related to knowledge of guidelines and current practice. Specific key guideline recommendations were compared to practice in two groups of surgeons: (a) those with a copy of the guidelines, and (b) those without. Results of questionnaires were discussed at a regional meeting with an external contributor.

Results: Twelve surgeons from eight hospitals performed 196 thyroidectomies. All participated in the study. Six surgeons were members of BAES and had a copy of the guidelines. One nonmember also had a copy.

Comparison of management in surgeons with and without national guidelines

	Guidelines	No guidelines	Fisher's test
Preoperative calcium	76 (88%)	14 (47%)	$P < 0.0001$
Postoperative calcium	56 (93%)	15 (100%)	NS
Fine needle aspiration	84 (68%)	12 (40%)	$P = 0.0064$
Recurrent laryngeal nerve	191 (98%)	34 (67%)	$P < 0.0001$
Isotope scan	11 (13%)	34 (30%)	$P = 0.048$

Percentage of those with/without guidelines complying with recommendation is shown.

Conclusions: This study has demonstrated significant variation in surgical practice between those with access to national guidelines and those without. For guidelines to be effective, all surgeons undertaking endocrine surgery must have access to them. Provision of guidelines forms one part of the audit process.

Audit 05

Late presentation to hospital continues to be the major cause of testicular loss following torsion

M.I.A. Mansoor, L.E. Hunt and S.W. Hosking

Department of Surgery, Poole Hospital NHS Trust, Poole, Dorset

Aims: Our previous audit of testicular torsion showed late presentation to hospital as the major cause of testicular loss in patients with torsion. Two years after issuing referral guidelines to local GPs, a reaudit was undertaken to determine if referral practice had changed.

Methods: A retrospective case note review of all patients who underwent scrotal exploration for testicular pain as an emergency between June 1999 and June 2001 was undertaken. The method was identical to our first audit.

Results: Forty-four patients were identified, of whom 14 had torsion. Twenty-eight patients presented to their GP initially; nine of these had torsion, and five (56 per cent) required orchidectomy. The median time to presentation after onset of testicular pain was 12 h (range 6–120 h; CI: 95 per cent). Sixteen patients presented to A/E; of these five had torsion and one (20 per cent) required orchidectomy. The median time to presentation was 8 h (range 4–72 h; CI: 95 per cent). These results are almost unchanged from our first audit. All patients were operated on within 6 h of admission to hospital.

Source of referral in relation to symptom duration

Time of presentation after onset of pain	A/E (number)	A/E (%)	GP (number)	GP (%)	Total
<6 h	7	70	3	30	10
6–12 h	4	24	13	76	17
>12 h	5	29	12	71	17
Total	16		28		44

Conclusions: Late presentation to hospital of patients with pain is still the chief cause of testicular loss from torsion. GP guidelines alone appear insufficient; improved health education leading to prompt self-referral is also required.

Audit 06

Most gastrointestinal cancers are diagnosed outside of the '2-week wait' referral guidelines

E.C. Makin, A.D.N. Scott, J.S. Jameson and I.M. Loftus

Department of Surgery, Glenfield Hospital NHS Trust, Leicester, UK

Aim: The 2-week wait fast-track referral guidelines for suspected GI cancers were introduced in July 2000. The aim of this study was to assess the cancer diagnosis rate within the 2-week wait system compared to conventional referrals 1 year after their implementation.

Methods: A prospective observational study of all patients referred to an endoscopy unit during a calendar month.

Results: A total of 144 lower GI and 177 upper GI examinations were performed during the study period, of which 28 were 2-week wait referrals (14 lower GI and 14 upper GI). Sixteen colorectal and five upper GI cancers were diagnosed, mostly outside of the 2-week wait system as shown in the following Table.

	2-week wait referrals	Other referrals
Colorectal cancers	4/14	12/130
Upper GI cancers	1/14	4/163
Total	5/28	16/293

Conclusion: The majority of patients diagnosed with GI malignancies are not identified according to the 2-week wait criteria. The criteria for the 2-week wait referral may need to be reassessed in the light of these results.

Audit 07

The surgical risk scale as an improved tool for risk-adjusted analysis in comparative surgical audit

R. Sutton, S. Bann, S. Sarin and M. Brooks

Department of Surgery, Watford General Hospital, Vicarage Road, Watford, Hertfordshire, UK

Aims: Comparative surgical audit is increasingly important and although methods have been proposed to allow for meaningful comparisons of patient outcome, none have been described without faults, making this comparison flawed or overtly complicated. An alternative risk scoring system incorporating the CEPOD grade (confidential enquiry into perioperative deaths), the ASA grade (American Society of Anesthesiologists) and the BUPA operative grade was formulated and assessed.

Methods: Prospective audit of 4308 patients admitted under the care of three surgeons between May 1997 and October 1999, creating an initial data set of 3144 procedures with 134 deaths. Each procedure allocated a score on the basis of the CEPOD, BUPA and ASA grade to devise a surgical risk scale (SRS) by adding together the values of the three variables, generating a scale ranging from 3 to 14. Multivariate logistic regression analysis involving the three variables and univariate analysis of the SRS score were undertaken. Receiver operating characteristic and calibration curves were formulated. This process was validated on another data set (2780 patients) derived from all admissions to the same surgeons between November 1999 and December 2000.

Results: Univariate logistic analysis of the SRS score revealed it to be significantly predictive of death ($b = 0.84$, $P < 0.001$) and did not overpredict mortality for low-risk procedures.

Conclusions: The SRS is easy to use, formulate and interpret. It provides an accurate prediction of mortality in general surgical patients across the entire 'risk' spectrum.

Audit 08

How bright are surgeons and their X-ray viewing practice?

B.N. Appleton, J.A. Gostling, G.R.J.C. Blackshaw and W.G. Lewis

Institution Department of Surgery, Royal Gwent Hospital, Newport, Wales, UK

Aims: Despite the wide assumption that an X-ray viewing box provides the ideal illumination, doctors on post take ward rounds frequently use any available light to view X-rays, arguably resulting in errors of interpretation. We assessed the strength of agreement between the interpretation of emergency X-rays by the duty surgical team, utilizing the nearest light (ceiling fluorescent tube or window), with that of the consultant radiologist's definitive X-ray report (utilizing light box).

Methods: The abdominal (AXR) and chest (CXR) X-rays of 100 consecutive patients admitted as surgical emergencies, and necessitating emergency radiology, were studied prospectively. The strengths of agreement between the surgeons' interpretations and the radiologists' reports (taken as 'gold standard') were determined using the weighted kappa statistics (κ_w). Disagreements were chased and clinical relevance assessed

Results:

	AXR	CXR
Sensitivity (%)	95	75
Specificity (%)	79	97
Positive predictive value (%)	75	86
Negative predictive value (%)	96	93
κ_w (95% CI)	0·71 (0·51–0·91)	0·75 (0·48–1·0)

Surgeons performed well, kappa statistics showing excellent agreement with radiologists' interpretations The major difference in AXR findings was the identification of faecal loading, which surgeons diagnosed more often Treatment of these 'false positives' often yielded an excellent clinical result For CXR interpretation, radiologists found more pathology than surgeons, but none were found to be relevant and, when a surgeon identified an abnormality, it was almost universally confirmed on reporting.

Conclusion: Surgeons on a busy ward round utilizing any light source match radiologists using an X-ray box when assessing radiological findings. The routine reporting of any emergency X-ray (and resultant loss from ward in many cases) may not be necessary.

Audit 09

Management of recurrent inguinal hernia is not N.I.C.E.

S.K Richards, J.J. Earnshaw and M.N. Vipond

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Aims: There is little good evidence available on the management of recurrent inguinal hernias, despite this, the National Institute of Clinical Excellence recently suggested that laparoscopic repair is superior. This study was a review of recurrent hernia repairs performed in a district general hospital by six consultant surgeons

Methods: A case note review was performed of all inguinal hernia recurrences from 1991 to 2001 inclusive. Some 171 cases were examined, 92 per cent of those identified on database The average age at recurrence was 64 years and 4 per cent were women. Some 49 per cent of the original operations had been performed locally and primary operation notes were available. In these, the original repair type was open nylon repair in 54 per cent, open mesh repair in 18 per cent and laparoscopic repair in 8 per cent

Results: Recurrence was seen more frequently on the right (61 per cent) than the left (39 per cent). Hernia recurrence was managed by open mesh technique in 63 per cent and laparoscopically in 22 per cent of patients. Complication rates were highest after emergency repair – all of these hernias were repaired by open methods with a complication rate of 50 per cent and one death In elective repair, complication rates were similar after mesh repair (15 per cent) and laparoscopic repair (11 per cent) The length of stay in the two groups was comparable at 1·2 and 1·3 days, respectively A single recurrence was seen in each group

Conclusions: This 10-year experience demonstrates equivalent results for laparoscopic or open repair of recurrent hernias refuting current N I C E. guidelines. A large randomized controlled trial is necessary to compare methods of repair of recurrent inguinal hernia

ASGBI: Colorectal 23–30

Friday, 24 May 08.30–10.00

Colorectal 23

Impact of surgery on growth and development in children with inflammatory bowel disease

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Aims: Inflammatory bowel disease (IBD) in children is associated with significant growth retardation. We have assessed the effects of surgery on height and weight velocity in children with IBD.

Methods: We have prospectively collected data for nine children (2 F, 7 M) with a mean age of 13.25 years (range 6.9–15.3 years) refractory to medical management for IBD. This group (seven Crohn's and two ulcerative colitis) had IBD for a mean duration of 43 months (range 4–72 months) prior to surgery. Height and weight velocity was calculated from measurements recorded at intervals 6 months prior to, at the time of and 6 months following surgery. All subjects were being treated with systemic/topical steroid or azathioprine/6MP or cyclosporine for at least 6 months before surgery.

Results: There was a significant change in both height and weight velocities following surgical intervention (see Table).

$n = 9$	Height (cm month^{-1})	Weight (kg month^{-1})
Mean preop velocity	0.19	−0.05
Mean postop velocity	0.52	0.74
Velocity change	0.33	0.79
Confidence interval	0.11–0.52	0.17–1.41
Significance	<0.01*	<0.05*

*Paired t-test.

Conclusions: At present, surgical intervention in children with IBD is used as a last resort measure despite compromise in growth and development. These data show a significant improvement in growth rates following surgery suggesting that early surgical intervention may be more appropriate in children with IBD.

Colorectal 24

Detection of circulating tumour cells at 24 h after primary resection predicts colorectal cancer recurrence

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Background: Recurrence develops in 35 per cent of node negative, but not in 32 per cent of node positive colorectal cancer patients and thus use of node status to identify patients for chemotherapy is uncertain. Circulating tumour cell (CTC) detection at 24 h after primary resection is associated with adverse tumour stage.

Aims: To determine whether CTCs are significantly associated with recurrence and whether addition of CTC information improves the accuracy of node status in predicting recurrence.

Methods: At 24 h after primary tumour resection, 3 × 14 mL blood samples were taken through preflushed cannulae. Following RNA extraction, RT-PCR was performed to detect carcinoembryonic antigen (CEA) and cytokeratin 20 (CK20) mRNA. A positive result required either to be positive in any of the three samples. Patients were followed up for a median of 922 days (SD = 712–1307) by outpatient clinical examination with serum CEA measurement and 6-monthly abdominal ultrasound scanning.

Results:

	Node status ($n = 128$ patients)		CTC status ($n = 99$ patients)	
	Negative Dukes' A + B	Positive Dukes' C	Negative	Positive
No recurrence	62	15 $P < 0.0001$	50	10 $P < 0.0001$
Recurrence	11	40	31	8

Both node status and CTC information was available for 96 patients (38 developing recurrence). Logistic regression analysis selected CTC positivity before node status as a recurrence predictor ($P < 0.0001$). Node positivity identified 30/38 (79 per cent; 95 per cent CI = 66–92 per cent) of patients developing recurrence. The use of either CTC or node positivity as a recurrence predictor increased this to 37/38 (97 per cent; 95 per cent CI = 92–100 per cent).

Conclusion: CTC detection at 24 h after primary colorectal cancer resection predicted disease recurrence. Addition of CTC information increased the accuracy of primary tumour node status in identifying patients requiring chemotherapy.

Colorectal 25

Endosonography and anal manometry assessment of third-degree tears and correlation with symptoms of incontinence

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Aims: To assess anal sphincter structure and functional outcomes following third-degree perineal tears and the effect of its disruption on the development of anal incontinence.

Methods: Fifty-one consecutive patients, 41 primigravidae and 10 multigravidae, with third-degree intrapartum perineal tears, primarily repaired, were recruited 4 months postpartum. Patients completed questionnaires assessing faecal incontinence. Anal manometry and endosonography were then performed.

Results: No patient reported incontinence prior to pregnancy. The incidence of faecal incontinence was 12 per cent. Six patients were incontinent of faeces and 10 incontinent of either flatus and/or fluid. Eighty-one per cent of anal incontinent patients were primigravidae (OR 0.96). Three women had previous third-degree tears, with one anal incontinence on subsequent delivery. Disruption of the external anal sphincter (EAS) was identified endosonographically in 26 patients. In 13 patients, there was an associated internal anal sphincter (IAS) defect. Clinically unsuspected tears of the Anal sphincter mechanism were diagnosed by anal endosonography in 46 per cent of asymptomatic patients. The EAS alone was involved in 14, IAS in three, and both in five patients. Clinical examination of women with anal incontinence identified an anal sphincter defect in 46 per cent. Ultrasound in anal incontinent patients showed an IAS defect in 42 per cent and an EAS defect in 52 per cent. All patients with faecal incontinence had a disruption of the EAS identified by endosonography ($P < 0.05$). No significant relationship was demonstrated between symptoms and anal manometry ($P = 1.0$, n.s.).

Conclusions: Third-degree tears cause significant, although clinically unsuspected anal sphincter defects. It may be that anal manometry alone is not sufficient to exclude sphincter injury.

Colorectal 26

Open access colonoscopy and radiology service: an alternative to specialist clinic for fast track referrals?

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Aims: Government guidelines stipulate that by July 2000 everyone with suspected colorectal cancer (CRC) will be able to see a specialist within two weeks of their GP referral. Open access barium enema (BE) and colonoscopy for patients fitting the criteria for fast track referral has been suggested as a potential solution to relieve pressure on specialist clinics

Methods: Between March 2000 and December 2001, data were prospectively collected on all patients seen in the colorectal specialist clinic via fast track referrals. None of the patients in our study were referred directly for BE or colonoscopy

Results: A total of 420 patients were seen in the clinic Fifty-one patients (12 per cent) were found to have CRC, 113 (26 9 per cent) had other conditions considered differential diagnoses of CRC. In 55 (12 6 per cent) patients significant other pathologies unrelated to the large bowel were identified. Of those patients, 14.5 per cent had a gynaecological malignancy, 7 2 per cent had prostate cancer and a further 14 5 per cent had other forms of malignancy. A total of 27.7 per cent of patients with noncolorectal diagnoses presented with medical problems, four of which necessitated urgent admission These pathologies were not diagnosed on BE or colonoscopy; 49 per cent were diagnosed at their clinic visit

Conclusion: There are a large number of inappropriate referrals through the fast track referral system and only 37 per cent of all CRC were referred via this pathway. The experience in our institution suggests that the referral criteria for the fast track system are neither specific nor sensitive and our data suggest that it is not safe to offer an open access colonoscopy and radiology service as an alternative to a specialist clinic.

Colorectal 27

Time for open access colonoscopy? A retrospective study comparing diagnostic yield from GP colonoscopy referrals with colonoscopy referrals from outpatient clinics

H El-Wakeel and T H. Walsh

St Mary's Hospital, Isle of Wight, UK

Aims: The number of colonoscopies performed is increasing, raising the question of whether a more open access approach to colonoscopy is desirable

Methods: A retrospective comparison of consecutive colonoscopy referrals for similar indications during 1998, from a rapid access colonoscopy clinic (RACC, $n = 100$) and outpatient clinics (OPC; $n = 100$) Data collected; referral indications, diagnosis and complications

Results: RACC: 82 per cent had a colonoscopy. In 3 per cent, a barium enema was considered appropriate and 15 per cent were not considered as needing colonic investigation Of the 15 per cent, none had a colonoscopy nor was colonic pathology diagnosed subsequently. Overall, the positive diagnostic yield was 52 per cent (diverticular disease 31 per cent, inflammatory bowel disease 10 per cent, polyp 7 per cent, colorectal cancer 4 per cent). Rate of completion was 91.5 per cent OPC. all underwent colonoscopy. The positive diagnostic yield was 52 per cent (diverticular disease 23 per cent, inflammatory bowel disease 7 per cent, polyp 15 per cent, colorectal cancer 6 per cent, 'other' 1 per cent) Rate of completion was 95 per cent There were no complications requiring hospitalization in either group.

Conclusions: Indications for referral and diagnostic yield were comparable to those in the literature, with no difference in diagnostic yields between the two groups (confidence interval 95 per cent = 44.8–59 1 per cent) There was no significant difference in positive findings between the two groups (diverticular disease $P = 0\ 20$, inflammatory bowel disease $P = 0.45$, polyp $P = 0\ 07$, colorectal cancer $P = 0\ 52$). Detailed outpatient assessment of GP colonoscopy referrals does not improve the diagnostic yield nor reduce the complications, and together with evidence from the literature, this supports the argument for moving towards open access colonoscopy.

Colorectal 28

Early feeding post major colonic surgery: why we should all be doing it

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Aims: Gastrointestinal surgery results in increased intestinal permeability increasing morbidity and mortality This study focuses on very early feeding post major colonic resection and how it influences outcome

Methods: Sixty-seven consecutive patients undergoing colonic resection were entered into the study Patients in the early feeding group (43) were allowed free fluids (plus external supplements) immediately following surgery Patients in the control group were treated in the 'traditional' fashion of awaiting resolution of bowel sounds and passage of flatus before free fluids were commenced There were 30 women and 37 men aged 26–90 (median 67) years

Results: The two groups were comparable by age, sex ratio, CEPOD status and ASA grade Patients in the 'early' group were tolerating free fluids at 0 65 (0–2) days compared to the 'traditional' group at 2 17 (1–9) days ($P < 0.001$) Time to 'normal' diet was 3.42 (0–9) days in the 'early' group and 5.58 (2–15) days in the 'traditional' group ($P < 0.001$) Complications included four major (including one leak, 1 MI) and three minor in the 'early' group and eight major (includign three leaks, 1 MI) in the 'traditional' group ($P = 0.127$) The 'early' group had one death from systemic sepsis, and there were two deaths from systemic sepsis in the 'traditional' group ($P = 0\ 045$). Time to discharge was 14 (5–44) days in the 'early' group and 22 (7–73) days in the late group ($P = 0\ 006$)

Conclusions: Early feeding by the enteral route reduces septic complications, inpatient mortality and significantly reduces inpatient stay, all of which have significant economic benefits

Colorectal 29

Development of a molecular metastasis index for colorectal cancer

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Aims: A significant proportion of colorectal cancer patients undergoing curative surgery develop treatment failure despite the absence of microscopic metastases in their lymph nodes (LN). Use of quantitative real-time RT-PCR allowed the development of a novel, more sensitive molecular metastasis index (MI)

Methods: This study was approved by the local Research Ethics Committee with informed consent obtained from the patient prior to surgery Total RNA was prepared from lymph nodes after surgery for non-malignant ($n = 17$ patients, 143 LN) or malignant disease ($n = 48$ patients, 448 LN, 30 of which were histologically positive) The 5′-nuclease RT-PCR assays were performed in triplicate, on an ABI Prism 7700 detecting cytokeratin 20 (ck20), carcinoembryonic antigen (CEA) and guanylyl cyclase C (GCC) mRNA. Absolute mRNA copy numbers were calculated using amplicon-specific standard curves

Results: *Specificity* In patients with benign disease, GCC, ck20 and CEA were detected in 17, 76 and 124/143 LN, respectively *Sensitivity*. In patients with histologically involved LN, GCC, ck20 and CEA were detected in 29, 28 and 30/30 LN, respectively There was good correlation between CEA and GCC mRNA copy numbers above a threshold level The mRNA copy numbers were used to develop a formula establishing MI with 99 per cent specificity and 97 per cent sensitivity which detected LN metastases in an additional 8/32 Dukes A/B patients

Conclusion: The MI can reliably identify LN likely to harbour metastases. Whether it can also predict metastatic potential to identify the patients most at risk of treatment failure requires longer follow-up.

Colorectal 30

Major colorectal cancer surgery: are trainees good enough?

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Aims: The introduction of Calman training several years has led to shorter training periods and concern by some that trainees are perhaps not as experienced as they once used to be. This study is designed to look at the outcome of major colorectal cancer surgery based on the grade of surgeon performing the procedure.

Methods: Data was prospectively collected onto a computerized database from mid-1994 until June 2001 for all patients undergoing colorectal cancer resection. A total of 343 cases (55 per cent male) with a median age of 71 (32–93) years were included. Operations were performed with the consultant as lead surgeon in 195 cases, supervised higher surgical trainee (HST) in 56 cases, and unsupervised HST in 92 cases.

Results: Unsupervised HST were more likely to perform right colonic surgery ($P < 0.001$), operate on the patients over 80 ($P = 0.004$) and never operated on patients having radiotherapy. Consultants were more likely to raise a covering stoma ($P = 0.003$), had more major complications ($P = 0.07$) and fewer long-term survivors ($P = 0.035$). There was no significant difference in leak rates, inpatient deaths, and local recurrence rates between consultants and HSTs. Five-year survival rates were similar for all groups when comparing survival curves, with no significant difference between consultant (55.7 per cent) and supervised HST (78.7 per cent) ($P = 0.09$) and consultant and unsupervised HST (57.8 per cent) ($P = 0.3$).

Conclusions: Apart from some minor differences that can be explained by slightly different case mix trainees performed as well as consultants for major colorectal cancer resection. The concerns are therefore not justified.

Six of the Best, Vascular 20

Trouble with short saphenous surgery

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Aims: In this prospective study, we attempted to explain the reasons for the high failure rates of venous surgery at the popliteal fossa, and the ideal place for the skin incision for short saphenous ligation.

Methods: Eighty-nine consecutive patients (100 limbs) presenting with suspected primary short saphenous varicose veins had their popliteal fossa assessed by duplex ultrasound and the sapheno-popliteal junction located and marked relative to the skin crease

Results: In 34 per cent of patients, either the giacomini or the gastrocnemius vein was incompetent rather than the short saphenous vein, furthermore in 9 per cent of patients the short saphenous vein terminated either with the giacomini or the gastrocnemius vein rather than the popliteal vein In 87 per cent of the cases, the sapheno-popliteal junction was found to be within 6 cm of the skin crease, but in 8 per cent of cases the sapheno-popliteal junction could not be identified.

Conclusions: A significant proportion of patients with primary short saphenous varicose veins had variable venous anatomy and sites of reflux at the popliteal fossa, accounting for the potential for inadequate surgery at this site and hence high rates of recurrence, requiring mandatory preoperative duplex assessment. In the majority of cases, a transverse incision placed at the site of the sapheno-popliteal junction is adequate, however, in a few cases the transverse incision can lead to difficulty in accurately ligating the sapheno-popliteal junction, an S-shaped incision may become necessary

Six of the Best, Vascular 21

A 1-year prospective study of management and outcome of patients presenting with critical lower limb ischaemia

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Aims: To determine management of patients with critical lower limb ischaemia (CLI) from first presentation to investigation and treatment

Methods: One-year prospective survey (May 2000–May 2001) Follow-up 3–15 months.

Results: Some 873 arterial cases presented, 134 patients had CLI Of the latter, 49 per cent were men, 30 per cent diabetic, the median age was 81 years. Only 15 (24 per cent) of the 62 cases were referred to outpatients as urgent. Vascular consultants reviewed all GP referral letters and the patients were prioritized Significantly more patients were prioritized as soon or urgent by vascular consultant surgeons that by GPs ($P < 0.001$). Patients waited a median of 25 days to be seen in outpatients, and had symptoms for a median of 8 weeks. Treatment was conservative for 70 patients, and 11 primary amputations, 6 secondary amputations, and 62 revascularization procedures (34 operative, 28 percutaneous transluminal angioplasty) were performed At follow-up (3–15 months, median 9 months), rates of major amputation and death were 12 and 27 per cent, respectively. Significantly more diabetics underwent major amputation ($P < 0.02$) than nondiabetics Patients presenting with ulceration or gangrene were at greater risk of death than those with rest pain alone ($P < 0.01$)

Conclusion: Patients with CLI often have symptoms for many weeks before being seen by a specialist, and 76 per cent are referred as nonurgent cases This compares with patients with suspected malignant disease who are required to be seen within 2 weeks

Six of the Best, Vascular 22

Complications of intervention in intermittent claudication

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Introduction: One of the arguments for a conservative policy in intermittent claudication is the potential risk of significant complications as a result of interventional treatment. The in-hospital complications of an interventional policy have been assessed for a 5-year period

Aims: All admissions for intermittent claudication between January 1997 and December 2001 have been analysed Data were entered prospectively in a dedicated database by a single observer. Data were collected on indications for admission, operative procedures and complications.

Results: A total of 1115 admissions took place in 531 patients In 35 admissions, no procedures took place A total of 569 admissions were for diagnostic arteriography, of which three had cardiac complications, one an allergic reaction and one a groin haematoma A total of 328 admissions were for angioplasty on a total of 352 vessels. Angioplasty complications included one TIA, one damaged vessel, one severe angina and one distal embolization which required operative intervention A total of 183 admissions involved reconstructive surgery Procedures included 19 aortic grafts, 73 above knee and 30 below knee femoro-popliteal bypasses, 14 distal grafts, 34 cross-over grafts and 17 assorted other bypasses. Complications of surgery included 12 assorted cardiac, respiratory and renal complications, 15 significant wound complications, two anastamotic bleeds/graft ruptures and seven graft thromboses Eight patients required further intervention in the same admission There have been no deaths and only one late amputation.

Conclusion: Radiological investigation and intervention in intermittent claudication should be regarded as a safe procedure but surgery carries a higher risk. Potential amputation should not be regarded as reason for non-intervention

Six of the Best, Vascular 23

Epidural analgesia is associated with a decreased mortality in elective abdominal aortic aneurysm (AAA) surgery

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[2]*Department of Anaesthetics, The Royal London Hospital, London, UK*

Aims: This study aims to evaluate whether addition of epidural analgesia in the peri-operative period is associated with a reduction of in-hospital mortality following elective abdominal aortic aneurysm (AAA) repair

Methods: Patients admitted to ICU following elective repair of AAA were identified from a London Regional Health Authority ICU Audit Prospective data were gathered from 20 ITU units over 9 years. Multiple logistic regression analysis was undertaken to identify putative predictors of survival following admission to the ICU. These included age, sex, chronic health score, acute physiological score (APS), intubation status and epidural analgesia

Results: There were 1289 patients in the database In this selected population, the in-hospital mortality with an epidural was 7.8 per cent ($n = 776$) *versus* 13.8 per cent ($n = 304$) without an epidural. The following factors were identified as independent predictors of mortality (adjusted odds ratios (OR) shown with 95 per cent confidence intervals) increasing age (OR 1.045; CI 1.016–1.076), APS (OR 1.1, CI 1.06–1.4), intubation (OR 3.322, CI 2.119–5.208), and the absence of epidural (OR 1.73, CI: 1.128–2.656) These effects were not significantly altered by inclusion of the year of operation and the surgical unit involved in the statistical analysis

Conclusions: The use of epidural analgesia in this population of patients following elective AAA repair was associated with a significant reduction of in-hospital mortality. The use of epidural analgesia in patients undergoing elective AAA repair is recommended.

Six of the Best, Vascular 24

The role of intra-abdominal pressure in the outcome of aneurysm surgery: is 15 the magic number?

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Aims: Elevated intra-abdominal pressure (IAP) may cause widespread organ dysfunction (abdominal compartment syndrome) through effects on the respiratory, cardiac, renal and gastro-intestinal systems. The aim of this study was to document IAP following aneurysm surgery, and to determine the effect of IAH on the outcome.

Methods: The patient cohort comprised 66 patients undergoing infra-renal aneurysm repair (45 nonruptured (32 conventional, 1 death; 13 endovascular); 21 ruptured AAA, 8 deaths). IAP was quantified at the termination of the procedure and 24 h postoperatively. Physiological indices of organ function were recorded, as were the levels of TNF-α and IL-6.

Results: IAP was significantly higher at abdominal closure following ruptured aneurysm repair (15.4 mmHg [SE 1.6]) than conventional (10.5 [0.89]) or endovascular repair (6.4 [1.0]) of nonruptured AAA. The sensitivity and specificity of IAP to predict subsequent mortality was analysed using a receiver characteristic operating curve. This analysis demonstrated that a cut off of 15 mmHg was most useful for indicating patients at risk (sensitivity: 0.66; specificity: 0.79). Physiological indices of organ dysfunction (pH [0.027], base excess [0.005], peak inspiratory pressure [0.0015], CVP and urine output [0.0029]) were significantly impaired in patients with $IAP > 15$ mmHg in comparison to patients with lower pressures. Similarly, IAP correlated significantly with the concentrations of IL-6 (0.0075) and TNF-α (0.0025), as well as with indices of cardiac (CVP 0.038), respiratory ($PaO_2/FiO_2 = 0.026$), and renal function (urine output = 0.046).

Conclusions: These data suggest that the management of IAP may have a role following repair of ruptured AAA.

Six of the Best, Vascular 25

Eleven-year experience with anatomical and extra-anatomical repair of mycotic aortic aneurysms

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Aims: Mycotic aortic aneurysms present a surgical challenge. We reviewed our management of both ruptured and nonruptured, abdominal and thoracoabdominal mycotic aneurysms in order to determine the safety and efficacy of *in situ* and extra-anatomical prosthetic repairs.

Methods: Data regarding presenting symptoms, investigations, operative techniques and outcome were prospectively collected on 15 consecutive patients treated at a single centre from 1991 to 2001 inclusive.

Results: There were 11 men and 4 women, with a median age of 70 years (range: 24–79). The median duration of symptoms was 26 days (range: 1–1810). All but one patient were symptomatic. Nine had back pain, five were pyrexial, six had a raised white cell count, six were anaemic, four were septic and six had a contained leak on admission. Blood or tissue cultures isolated *Salmonella* ($n = 3$), staphylococci ($n = 3$), streptococci ($n = 1$), coliforms ($n = 1$), *Treponema pallidum* ($n = 1$), while in six patients no organisms were identified. Preoperative CT identified four infra-renal, four juxta-renal, three (Crawford thoracoabdominal) type IV, three type III and one type II aortic aneurysms. Thirteen were repaired with in situ prostheses following extensive local debridement and two required axillo-femoral prosthetic grafts. There were four early deaths, two (type IV) relating to blood loss, one secondary to myocardial event (type IV) and one secondary to multiorgan failure (juxta-renal). All surviving patients have been followed up for a median duration of 38 months (range: 1/2–112 months). There were two late deaths at 3 months (juxta-renal) and at 2 years (type III), the latter relating to graft infection.

Conclusions: In the absence of uncontrolled sepsis, *in situ* repair of mycotic aortic aneurysms using prosthetic grafts achieves durable results with only one late infection.

ASGBI: Breast 13–24 Friday, 24 May 13.45–15.45

Breast 13

Why do screen age women (50–64 years) with breast cancer present to a symptomatic clinic?

S. Ghosh

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Aims: In Wales National Breast Screening Programme, diagnosis and treatment of screen-detected breast cancers are undertaken centrally, although most DGHs have a symptomatic breast clinic. An analysis of screen age patients with primary breast cancers presenting to symptomatic clinic is undertaken.

Methods: All patients of ages between 51 and 65 years presenting with primary breast cancer in the symptomatic breast clinic of a Welsh DGH between 1997 and 2001 were analysed. Breast screening records, if any, of these patients were obtained from the screening programme.

Results: A total of 105 patients were identified. Of these 16 (15.2 per cent) were never invited for screening. Eighteen (17.1 per cent) although invited never attended screening. Twelve (11.4 per cent) lapsed after screening more than 3 years back. Fifty-nine patients (56.2 per cent) had been recently screened. Eleven (18.6 per cent) of these were screened in the last 12 months, 25 (42.4 per cent) in the last 12–24 months and a further 23 (39 per cent) in the last 24–36 months.

Conclusion: One would have expected that in a symptomatic clinic, the majority of patients presenting with breast cancer in the screen age would have been women who never attended screening, but this group was only 17.1 per cent of the total. The majority of the women (56.2 per cent) were interval cancers, significant number (18.6 per cent) of them were within the first year after screening. We were also concerned to find that a number of women (15.2 per cent) were missed by the system and were never invited into the screening programme.

Breast 14

Does screen-detected breast cancer has a better 5-year survival than symptomatic breast cancer?

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Aims: The aim of this study was to assess the 5-year survival for women with screen-detected breast cancer and to compare that with survival in women presenting with symptomatic breast cancer.

Methods: Prospectively collected data on patients diagnosed with invasive breast cancer between January 1993 and December 1994 and followed up until the end of 1999 was collated and analysed. Five-year survival was broken down by age at diagnosis, tumour size, grade and nodal status. The overall 5-year survival for women with screen-detected cancers was compared with that for women with symptomatically presenting cancers.

Results: A total of 308 patients with invasive breast cancer were referred to the unit (162 via the breast screening programme and 146 presenting symptomatically). The overall 5-year survival was 85.5 per cent (CI: 80.8–89.1). Small tumour size, low grade and negative nodal status were associated with higher survival rates. Five-year survival in the screen-detected patients (91.7 per cent; CI 85.8–95.2) was higher than in those presenting symptomatically (78.6, CI: 70.6–84.6, $P < 0.001$).

Conclusion: These findings suggest that patients with screen-detected breast cancer have better survival rates compared to those with symptomatically detected breast cancer. This supports the argument in favour of a beneficial impact of breast screening programmes on patients' survival.

Breast 15

Cyclooxygenase-2 (COX-2) is associated with lymphatic and haematogenous metastases in breast cancer

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Aims: COX-2 has been shown to be important in angiogenesis and carcinogenesis. We hypothesized that COX-2 expression in breast cancer would correlate with an invasive phenotype.

Methods: Forty-one patients with breast cancer had level 3 axillary dissection and bone marrow aspiration (from rib and iliac crest). Nodes were analysed by H and E staining, while marrow was assessed for micrometastases using flow cytometry and the APAAP technique. Primary tumours were studied for COX-2 expression and microvessel count. Microvessels were visualized by staining for CD34 antigen. For COX-2, goat anti-COX-2 antibody binding was visualized by incubating sections with biotinylated anti-goat antibody followed by streptavidin coupled to horseradish peroxidase using the VectaStain system, then incubated with 3,3′-diaminobenzidine to visualize antibody complexes. Microvessels were counted by two independent blinded observers. COX-2 staining was scored, and the percentage of positive cells calculated. Statistical analysis was performed using the SPSS package of Microsoft.

Results: COX-2 was expressed in 36/41 patients. There was a significant inverse correlation between microvessel count and COX-2 staining. Microvessel density did not correlate with tumour grade, stage, lymphatic metastases or bone marrow micrometastases. Conversely, the degree of COX-2 staining correlated significantly with the presence of lymph node metastases, but not with the number of nodes involved. Similarly, it correlated significantly with marrow micrometastases and tumour size, but did not correlate with stage or grade.

Conclusion: We have demonstrated that COX-2 is associated with lymphatic and haematogenous metastases in breast cancer, suggesting COX-2 inhibition as an effective adjuvant treatment in controlling metastases.

Breast 16

pMQ1 expression in human breast cancer: correlation with established prognostic factors

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Aims: pMQ1 is a tumour-associated glycoprotein with characteristics suggestive of an adhesion molecule. The level of pMQ1 expression had been positively correlated with increasing histological grade in astrocytomas. pMQ1 was also found to be expressed in metastatic breast cancer in the brain. The aim of this study was to determine the correlation between the level of pMQ1 expression and known prognostic indicators in breast cancer.

Method: Breast cancer ($n = 228$) were examined for pMQ1 expression, p53 and Ki-67 by the immunohistochemical method. The expression of pMQ1 was compared with known prognostic indicators (age, tumour size, histological grade, lymph node status, lymphovascular invasion, oestrogen receptor status, p53, Ki-67, Nottingham prognostic index and TNM-UICC staging) using a bivariate correlation analysis.

Results: pMQ1 was expressed in 60 per cent (137/228) of the breast cancers. The normal surrounding tissue margins were negative for pMQ1 expression, and benign breast diseases always lacked pMQ1 expression. A significant positive correlation was observed between pMQ1 expression and the tumours' histological grade ($P \leq 0.00005$), and lymphovascular invasion ($P = 0.018$). pMQ1 was inversely correlated with oestrogen receptor status ($P = 0.001$). However, no correlation exists between pMQ1 and age, tumour size and lymph node status. pMQ1 was significantly correlated with Nottingham prognostic index ($P = 0.008$) but not with TNM-UICC staging.

Conclusion: pMQ1 has a positive correlation with tumour histological grade, lymphovascular invasion and Nottingham prognostic index, and inverse correlation with oestrogen receptor status. Therefore, pMQ1 may have prognostic importance in identifying biologically aggressive breast tumours.

Breast 17

Deprivation and prognostic indicators in operable breast cancers

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Aims: Patients from deprived areas with breast cancer have a poorer outcome, the reasons for this are unclear. The aim of this study was to assess the influence of deprivation on various pathological prognostic factors in patients with operable breast cancers.

Methods: Prospective data were collected on all operable breast cancers treated in Glasgow between October 1995 and October 2001. They were separated into three groups – affluent, intermediate and deprived according to the Carstairs index of deprivation. The influence of deprivation on pathological size of the tumour, ER status, histological grade and axillary node status were examined.

Results: A total of 3251 patients were included, 598 affluent, 1473 intermediate and 1180 deprived. Patients in the deprived group were significantly more likely to have larger tumours, with 33.3 per cent of affluent, 35.6 per cent of intermediate and 43.4 per cent of deprived patients having tumours of >20 mm ($P < 0.001$). There was a higher incidence of patients in the deprived group with high-grade tumours, 27 per cent of affluent, 30 per cent of intermediate and 35 per cent of deprived patients had grade 3 tumours ($P < 0.01$). There was also a trend for the more deprived patients to have a higher incidence of ER-negative tumours. The deprived group were significantly less likely to have a highly positive (≥50 per cent) ER status ($P = 0.05$).

Conclusion: Patients from deprived backgrounds are significantly more likely to have larger tumours at operation. They are also more likely to have high-grade tumours and less favourable ER status. This may explain why patients from deprived areas have a poorer outcome.

Breast 18

Expression of cerbB-2 does not prevent the withdrawal response to hormone replacement therapy (HRT) in breast cancers

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University Hospital of South Manchester and [1]Paterson Institute for Cancer Research

Aims: The presence of cerbB-2 oncoprotein is said to predict failure to respond to endocrine therapy in breast cancer (particularly tamoxifen). The aim of the study was to determine whether the effect of oestrogen deprivation (by stopping HRT) on breast cancer is related to cerbB-2 oncoprotein expression.

Methods and study: We identified 129 women who were on HRT at the time of diagnosis of breast cancer by core biopsy. Out of 129 women, 114 stopped HRT and 15 continued HRT till surgery. Ki67 (a marker of cell proliferation), progesterone receptor (PR), ER (oestrogen receptor) and cyclin D1 (a cell cycle inducer) were measured by immunohistochemistry on paired sections of core biopsy and operative specimens. CerbB-2 was measured on the operative specimen and was scored from 0 to 3.

Results: Stopping HRT led to a significant fall in epithelial proliferation compared to continued group ($P = 0.05$), more so in ER positives ($P = 0.045$)* Mann–Whitney test. PR ($P = 0.007$)* and cyclin D1 ($P = 0.05$)* decreased significantly in responding tumours regardless of cerbB-2 status ($P = 0.498$, Kruskal–Wallis test).

Status of cerbB-2	*n*	Median Ki67 change	Cyclin D1
Negative: 0, 1	65, 33	1.74 (−7.8–12.02), 1.51 (−12–11.99)	HRT stopped −9.5 (−31.9 ± 14.3)
Positive: 2, 3	12, 4	1.20 (−3.7–7.51), 1.98 (−17.3–28.6)	HRT continued 3.97 (−4.6 ± 23.2)

Conclusion: CerbB-2 expression does not prevent fall in cell proliferation due to HRT withdrawal. Aromatase inhibitors may still be the optimal strategy for ER and cerbB-2-positive breast cancers.

Breast 19

Elastic scattering spectroscopy for the diagnosis of breast cancer

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Introduction: Elastic scattering spectroscopy (ESS) studies tissue structure by spectral analysis of light absorption and scattering by intra- and extracellular components of tissue, and has previously been shown to be sensitive to morphological changes in tissue. The aim of our study is to determine the accuracy of ESS in diagnosis of breast cancer.

Methods: ESS system consists of a white light source, a parallel pair of optical fibres, a spectrometer and a computer. The process involves the delivery of light from the light source directly onto breast tissue via an optical fibre. The light is collected by the second optical fibre, and is analysed by the spectrometer and the computer, generating an optical spectrum. The entire process takes less than 1 s. Using model base analysis, the optical spectrum was analysed against an independent set of optical spectra taken from normal and malignant breast tissue to provide the diagnosis. The result was compared to the histological findings of the breast tissue.

Result: Optical spectra were taken from 94 breast tissues. ESS provided correct diagnosis in 87 out of 88 breast tissues (six were deemed indeterminate). ESS was able to diagnose 15 out of 16 breast cancers. There was one false positive, thus giving a sensitivity of 93.8 per cent, specificity of 98.6 per cent, positive predictive value 93.8 per cent and negative predictive value 100 per cent.

Conclusion: ESS has the potential to provide an instant and nonoperator-dependent detection of breast cancer, which can be used to guide core biopsies and to assess breast lesions and tumour margins intraoperatively.

Breast 20

Risk factors for false-negative breast cytology

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Aims: Freehand fine needle aspiration cytology (FNAC) has a reported sensitivity of 65–98 per cent for breast cancers with the majority of false-negative results being due to sampling error by the clinician and a significant minority to interpretative error by the cytopathologist. The aim of this study was to investigate the significance of tumour characteristics on false-negative cytology.

Methods: A database of patients with invasive or *in situ* breast cancer in whom the initial cytology was either C1 (inadequate) or C2 (benign) between 1996 and 2000 was reviewed. The age of patients and tumour characteristics were compared with those with initial C5 (malignant) cytology during the same period. Patients with local recurrence, previous surgery or radiotherapy to the breast or with no histology were excluded.

Results: There were 82 patients with false-negative cytology and 329 tumours in 324 patients with C5 cytology. The mean age of the two groups was 59.8 years (C1/C2) *versus* 66.5 years (C5). The proportion of patients below age 40 years was 8.5 per cent *versus* 4.3 per cent. The mean tumour size was 2.2 cm (C1/C2) *versus* 2.4 cm (C5). However, for tumours of ≤ 1 cm in size, the respective proportions were 13.6 per cent *versus* 3.4 per cent. Lobular carcinomas constituted 30.5 per cent of the histological type in the false-negative cases, but only 8.5 per cent of those with malignant cytology.

Conclusion: False-negative cytology was more common in patients below age of 40 years, in tumours of <1 cm diameter and in lobular cancers.

Breast 21

Do older women get poorer treatment after breast-conserving surgery?

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Aims: Breast-conserving surgery (BCS) followed by radiotherapy has replaced mastectomy as standard treatment for small early breast cancers. Failure to give adjuvant radiotherapy leads to a threefold increase in local recurrence rates.

Methods: To determine the factors which determined oncology assessment and radiotherapy use following BCS, all women aged 60–70 years undergoing BCS in Greater Manchester (between 1997 and 1998) were identified and case notes reviewed. Assessment by an oncologist within 3 months or commencement of XRT within 6 months were considered appropriate management. All hospital units treated more than 50 breast cases per year.

Results: Out of 436 women identified, 343 (79 per cent) saw an oncologist of which 323 (74 per cent) were given XRT. Assessment rates varied from 61 to 94 per cent between hospital, but did not differ between deprivation quintiles. With increasing age, fewer women were sent for oncological assessment or XRT ($P < 0.001$). A significant difference in radiotherapy and assessment rate was seen (61–94 per cent) ($P > 0.01$).

Table 1 Assessment by oncologist

		Assessed by oncologist		XRT given	
Age group	*n*	<10 mm	>10 mm	<10 mm	>10 mm
60–64	182	40 (85%)	127 (95%)	37 (79%)	126 (94%)
65–69	95	14 (74%)	68 (91%)	13 (68%)	66 (88%)
70–79	159	6 (30%)	87 (62%)	4 (20%)	76 (58%)

$P < 0.001$

Conclusion: Despite the widespread use of guidelines, many elderly women do not get appropriate treatment after BCS. Since the majority of breast cancer deaths occur in the 60–80 years age group, adherence to protocols will be necessary to reduce mortality further.

Breast 22

Breast conservation surgery – a comparison between ductal and lobular carcinoma

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Aims: To compare the re-excision rate following wide local excisions in lobular cancers (ILC) and ductal cancers (IDC).

Methods: One hundred and fifty patients with a diagnosis of invasive lobular cancers were compared with 150 women with invasive ductal carcinomas during the same time period.

Results: The mean ages were similar: 59.6 years IDC *versus* 59.7 years ILC. Twenty-nine per cent of IDC and 32 per cent of ILC were screen detected. Pathological tumour sizes were T1, 101 *versus* 62, T2, 43 *versus* 65, T3, 0 *versus* 17 in IDC and ILC, respectively. Invasive lobular cancers were more likely to be T3 cancers ($P = 0.01$). Twelve patients in the IDC group and 30 patients in the ILC group had multifocal disease ($P = 0.001$). One hundred and twelve patients in the ductal group and 91 patients in the lobular group were treated by wide local excision. Six of the 112 patients (5.4 per cent) with IDC and 29 of 91 patients with ILC (32 per cent) needed further re-excisions for involved margins ($P < 0.001$). Twenty-six per cent patients in the ductal group and 55 per cent of the lobular group were treated ultimately with mastectomy ($P < 0.001$).

Conclusion: This study confirms that conservation surgery carries an increased risk of re-excision with an increase in the mastectomy rate in patients with invasive lobular carcinoma. Where patients are considered for breast conservation but the core biopsy shows invasive lobular carcinoma, they should be warned about the increased rate of re-excision and mastectomy.

Breast 23

Do primary tumour characteristics predict axillary nodal status in breast cancer?

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Aims: To determine whether primary tumour characteristics of breast cancer – size, histological type, grade, vascular invasion (VI) and receptor status (ER) correlate with axillary nodal involvement. Can we select a group of breast cancer patients in whom axillary surgery can be safely avoided?

Methods: A retrospective analysis of 1496 patients with breast cancer presenting between 1986 and 2001 was carried out. All patients had been treated by wide local excision/mastectomy as well as an axillary four-node sample/level-III clearance.

Results: Five hundred and sixty (37.4 per cent) out of a total 1496 patients had metastases in axillary nodes. The following table shows the lymph node (LN) involvement compared with size, grade and VI.

Size (cm)	LN involved (%)	Grade	LN involved (%)
0.0–0.5	5/35 (14.3)	I	87/349 (24.9)
>0.5–1.0	17/129 (13.2)	II	236/593 (39.8)
>1.0–2.0	176/584 (30.1)	III	149/309 (48.2)
>2.0–4.0	298/651 (45.8)	VI	
>4.0	64/97 (66.0)	Yes	268/483 (55.5)
		No	154/670 (23.0)

Univariate analysis showed VI [Pearson Chi-square (pcs): 128.1, df 2, $P < 0.0001$], size [pcs: 106.8, df 4; $P < 0.0001$], grade [pcs 39.9, df 2; $P < 0.0001$] and ER status [pcs 9.5, df 2; $P < 0.009$] to correlate positively with positive LN status in decreasing order of strength. However, multivariate analysis showed that only VI and size independently predict LN positivity. Even tumours less than 5 mm had nodal involvement in over 10 per cent of cases. Only those tumours less than 4.5 mm diameter were free of nodal metastases ($n = 22$).

Conclusions: Although axillary LN involvement correlated positively with size and vascular invasion, it would be unwise to presume negative axillary node status in any subgroup of invasive breast cancer.

Breast 24

Breast sepsis – a unit's experience

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Aims: Assess the impact and review the symptoms and management of those referred to a breast clinic with breast sepsis.

Methods: A retrospective review of all patients presenting with breast sepsis over a 3-year period. These were divided into lactational and nonlactational (nipple-areolar and peripheral). Data were retrieved from the unit's database and patients' notes.

Results: A total of 175 patients presented over this period (~2 per cent of breast unit referrals). Thirty-six (21 per cent) were lactational in origin. Twenty-eight (77 per cent) had a palpable lump. Thirty-three (92 per cent) underwent ultrasound, with 21 (64 per cent) having guided aspirations (two of these in the no-lump group). Twenty-one (58 per cent) of this group were smokers and two (6 per cent) diabetics. Three (8 per cent) underwent surgical drainage. The predominant organism was *S. aureus*. The median number of outpatient episodes was two (range 1–22). A total of 139 (79 per cent) were nonlactational in origin and 121 (87 per cent) had a palpable lump. One hundred and twenty-four (89 per cent) underwent ultrasound, with 88 (71 per cent) having guided aspirations (seven in the no-lump group). Sixty-eight (49 per cent) were smokers and nine (6 per cent) diabetics. Twelve (9 per cent) underwent a surgical procedure. The main pathogens were anaerobic. The median number of outpatient episodes was three (range 1–15).

Conclusion: Breast sepsis is a significant part of a unit's workload. The use of broad-spectrum antibiotics, ultrasound ± aspiration and specialist review is the basis to good management. Most patients can be managed on an outpatient basis, but surgery is sometimes indicated.

ASGBI: Clinical Practice 01–08

Friday, 24 May 13.45–15.45

Clinical Practice 01

Can the outcome of home parenteral nutrition (HPN) be improved?

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Introduction: HPN is a complex treatment for patients with intestinal failure who are unable to maintain fluid or nutritional status. A multi-professional team approach to management reduces complications, but in a dispersed population this expertise is not available in every hospital. A managed clinical network (MCN) has been established to improve patient care. Before this an informal group existed but not all hospitals were involved and there was no funding.
Aims: The audit performed by the network aims to assess outcome of HPN and thus change practice.
Methods: The MCN is a formally organized multi-professional group who have produced evidence-based protocols for HPN. Group members agree to follow the protocols and submit audit data on patient numbers, clinical details and complications to the network manager. Chi-square and Student's t-test were used to assess the differences between the years.
Results: Fourteen per million patients in Scotland receive HPN (8–10//million in England and Wales). Data from the year prior to the network and the current year are shown in Table 1.

Table 1

	2000	2001	
Total patients	40	72	
Total hospitals	6	15	
Underlying disease			
Crohn's	19	28	
Ischaemia	5	9	
Radiation enteritis	3	4	
Motility disorders	6	10	
Malignancy	3	7	
Other	4	14	
Patients admitted	23	29	$P < 0.1$
No. admissions	69	58	
Catheter sepsis rate per 1000 patient days	1.6	0.9	$P < 0.05$

Conclusions: The establishment of a formal MCN has led to involvement of more hospitals and patients. Fewer patients required admission on a smaller number of occasions last year. There has also been a reduction in the catheter sepsis rate. This may reflect the more widespread use of protocols.

Clinical Practice 02

Adverse events and intravenous fluids

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Aims: Over 50 per cent of adverse events suffered by hospital patients in the United Kingdom occur in general ward care, including the prescription of medications and intravenous fluids (IVF). We performed a prospective evaluation of IVF prescription for postoperative patients in a surgical unit.
Methods: Data were prospectively collected regarding fluid balance, electrolyte status, fluid prescription and adverse events for each postoperative day until IVF were discontinued. The first 24 h were excluded. Patients receiving parenteral nutrition were excluded.
Results: Thirty-nine postoperative patients representing 103 patient days were included. Total daily volume of fluid prescribed [median (interquartile range)] was 3000 mL (1000 mL) in elective patients and 2500 mL (1000 mL) in emergency patients. There was no correlation between daily volume prescribed and patient weight (correlation coefficient: −0.104; $P = 0.373$). Correlation between electrolyte prescriptions and serum electrolytes was also poor. There was an inverse correlation between total volume prescribed and total losses in the previous 24 h (correlation coefficient: −0.2; $P = 0.044$). Seven patients required fluid challenges for hypovolaemia after the first postoperative day, three developed fast atrial fibrillation and one developed congestive cardiac failure requiring transfer to a critical care area after receiving 4 L of crystalloid in the previous 24-h period. There were no perioperative deaths.
Conclusions: Fluid prescription is highly variable. It is not tailored to the individual patient and may lead to adverse events. The use of precalculated fluid regimens based on the patients' body mass index with additions for ongoing losses may improve current practice.

Clinical Practice 03

The use of Rt-Pa for thrombolysis in acute massive pulmonary embolism

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Aims: Conventional therapy for acute pulmonary embolism is associated with high mortality and poor outcome. Our aim was to ascertain the efficacy of thrombolyis with Rt-Pa in patients with acute massive pulmonary embolism.
Methods: Patients with pulmonary embolism and circulatory hypotension were recruited. Antegrade groin punctures were performed in these patients with 7 or 8 F introducers with guiding wires. The catheter was passed into the pulmonary artery, the diagnosis confirmed and mechanical agitation of the embolus performed. After an initial bolus of intrapulmonary Rt-Pa (3 mg), Rt-Pa was commenced at 2 mg h^{-1}. Repeat angiograms were performed at 12 and 24 h. Pulmonary artery pressure was measured prior to the commencement of lytic therapy and at the time of radiologically proven clot dissipation.
Results: Ten patients with angiographically proven pulmonary embolism underwent thrombolytic therapy (8 F and 2 M). Mean age was 56.5 years (22–80 years) with mean time to lysis 17 h (6–48 h). Average Rt-Pa dose was 34.5 mg (15–55 mg).

	MBP (mmHg)	MPAP (mmHg)	MPAP/MBP (mmHg)
Pre-lysis	64.3	37.9	0.58
Post-lysis	105.6	23	0.21

MPAP, mean pulmonary artery pressure.
MBP, mean systemic blood pressure.

There were two deaths. Both patients suffered asystolic arrests; the latter showed good radiological evidence of clot dissipation. There was a significant fall in mean pulmonary artery pressure ($P < 0.05$) with restoration of MPAP : MBP to normal parameters.
Conclusion: Our results indicate that intrapulmonary thrombolysis may have a valuable and significant role in the management of life-threatening pulmonary embolism. This technique allows for a substantially reduced dose of Rt-Pa and may be employed in postsurgical patients in whom full thrombolysis is contraindicated.

Clinical Practice 04

Surgical gloves: how do you change yours?

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Aims: Surgical gloves are frequently changed intraoperatively, and different techniques exist. We surveyed surgeons and nurses in our hospital and prospectively compared contamination with two popular methods.

Methods: Twenty-five surgeons and 25 nurses were questioned regarding their intraoperative glove changing technique. Twenty scrub staff performed a standard 'surgical scrub', following which aliquots of fluorescent powder were applied symmetrically to each forearm prior to donning gown and gloves. Subjects were randomized to group 1 (open glove change, followed by closed technique) or group 2 (closed glove change, followed by open technique). Following glove change, glove and hand contamination was assessed.

Results:

	Open technique	Closed technique	*P*-value
Surgeon preference	20	5	
Nurse preference	0	25	
Mean glove contamination (cm^2)	0.11 (SD = 0.31)	0.59 (SD = 0.53)	0.008
Mean hand contamination (cm^2)	16.63 (SD = 22.43)	50.25 (SD = 28.65)	0.003

Conclusions: There is a preference for glove-changing technique noted between surgeons and nurses. The 'closed' technique produced significantly more contamination and we therefore recommend the 'open' technique for intraoperative glove changing.

Clinical Practice 05

The influence of the basic surgical skills course on needlestick injuries

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Aims: To assess the impact of the basic surgical skills course (BSS) on glove perforation during simple suturing.

Methods: The study was conducted at a regional BSS course. Fifteen senior house officers attended. All participants were asked to place two simple sutures (Ethilon) in artificial skin whilst wearing surgical gloves (Bodyguard). After completion of this task, the gloves used were collected in bins marked dominant and non-dominant hand. Any perceived needlestick injuries were documented. Each participant performed this task three times. On the final day of the course, the suturing exercise was repeated three times and all gloves collected. Ninety gloves were tested after wearing and performing no task as control. All gloves were tested electronically for perforations.

Results: Pre-course, the perforation rate was 4.4 per cent (2/45) in the dominant hand, 11.1 per cent (5/45) in the non-dominant hand and 7.8 per cent (7/90) overall. Post-course, the perforation rate was 0 per cent (0/45) in the dominant hand, 2.2 per cent (1/45) in the non-dominant hand and 1.1 per cent (1/90) overall. No needlestick injuries were reported during the study. Control perforation rate was 1.1 per cent (1/90).

Conclusion: The BSS course significantly reduces ($P = 0.032$) the glove perforation rate whilst suturing. We conclude that the BSS course improves sharp awareness and feel formal teaching should be encouraged in other specialities where suturing occurs.

Clinical Practice 06

Is 'sub-specialization' a problem for the provision of a safe emergency 'general' surgery service

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Aims: Sub-specialization for elective surgery may de-skill 'general' surgeons, potentially reducing the safe care of general surgical emergencies. This study aimed to determine how often a mismatch occurs between a sub-specialist surgeon and major specialist emergency operations.

Methods: In a DGH with a population of 500 000, where all the surgeons are sub-specialized and with a separate vascular take, the mismatch between major emergency operations and the sub-speciality of surgeon on-call in a 1-year period was determined. The complexity of the surgery was determined according to BUPA classification.

Results: In 1 year, there were 4225 non-elective admissions, of whom 1554 had an emergency operation. A total of 266 were at least major or of greater complexity from which there were 65 mismatches between surgeons and major operations, as shown below.

Specialist major operations	Specialist (surgeons)			Operations mismatched
	UGI	LGI	Breast	
UGI	–	11	9	20
LGI	23	–	22	45
Breast	0	0	–	0
Surgeons mismatched	23	11	31	65

Sixty-nine per cent (45/65) of mismatches were between LGI (lower gastrointestinal) operations and UGI (upper gastrointestinal) and breast surgeons. Fifty-five per cent (11/20) of major UGI operations were for simple suture-perforated ulcer, 15 per cent (3/20) cholecystectomies, 20 per cent (4/20) under-running bleeding peptic ulcers and 10 per cent (2/20) gastrectomies. Seventy-eight per cent (51/65) mismatches occurred out of normal working hours of which 31 per cent (16/51) were done by consultants.

Conclusion: The major problem in the future will be for UGI and breast surgeons to maintain their skills in LGI surgery. However, mismatches occurring out of hours requiring consultant participation are uncommon.

Clinical Practice 07

Research in surgical training — when? — and for how long?

M.S. Javed, E. Harrison and I. Taylor

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Aims: To assess the views of the consultant surgical community regarding the role of research in surgical training post-Calman.

Methods: A postal survey of consultant members of ASGBI was performed to assess views on desirability, timing, duration and method of assessment of research in surgical training. The results were compared with a similar survey conducted pre-Calman in 1995.

Results: A 50 per cent response rate was achieved compared to 71 per cent in the 1995 survey. Eight-two per cent of respondents had themselves obtained a higher degree by thesis. Overall, 89.7 per cent thought that research in training was either essential or desirable, compared to 89 per cent in the previous survey. Only 1.4 per cent felt that research should be conducted during BST (Basic Surgical Training) compared to 8 per cent previously. Forty-seven per cent indicated that research should be performed between BST and HST (Higher Surgical Training). Thirty-six per cent felt research during HST was most appropriate. Sixty-one per cent felt 1 year and 36 per cent 2 years of research was sufficient (91 and 9 per cent, respectively, in 1995). A total of 27.5 per cent thought that a thesis was the most appropriate method of assessment (33 per cent previously), while 35 per cent were supportive of an MSc (33 per cent previously). Eighty per cent of consultants with a higher degree thought research carried out as a trainee was worthwhile, both for their own education and career. There were no significant differences based on either age or specialist interest of the respondents.

Conclusions: Even in the post-Calman era, 90 per cent of surgeons still regard research as either essential or desirable, although 1 year is regarded as adequate by the majority.

Clinical Practice 08

Deaths while in surgical care: mapping improvements in practice over 7 years of continuous peer review audit

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Aim: To map changes in practice over 7 years of continuous peer review audit of surgical deaths

Methods: Between 1994 and 2000, all patients who died under surgical care (mean 4504 patients per annum, range 4331–4657) were peer reviewed for the Scottish audit of surgical mortality (SASM).

Results: The mean age rose to 76 years, 88 per cent were emergency admissions, but the absolute number and proportion of deaths following elective admission fell to 0 11 per cent For operative cases, 80 per cent of fatalities had major co-morbidity Surgical adverse events were usually related to clinical decision making, not poor facilities (e.g. late referral resulting in delay to surgery in 68 per cent where delays were identified) but contributed to (2 per cent) or caused (1 per cent) the death of some patients Failure to use HDU (high dependency units), which was highlighted by SASM, peaked as an adverse factor in 1998 and has since declined to 1996 levels due to increased facilities A consultant decision to operate rose to 97 per cent, consultant input as operator/assistant increased to 76 per cent ($P = 0.0001$, Chi-square test) and criticisms of surgeon grade and choice of operation declined to 1.7 per cent of deaths by 2000 There was almost a doubling of consultant anaesthetic input to 90 per cent by 2000 ($P < 0\ 0001$, Chi-square test)

Conclusion: Despite an increasingly elderly population, often admitted as an emergency, SASM has mapped significant changes in practice for patients who die, including the use of HDU, which may reflect overall improvements in the service

ASGBI: Posters 01–80

Lower Gastrointestinal

Poster 01

Neuromodulation as a new surgical treatment for faecal incontinence due to scleroderma

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Aims: Faecal incontinence occurs in over one-third of patients with scleroderma, has a multifactorial pathogenesis, and is often resistant to conventional treatment. Sacral nerve stimulation is a novel treatment for faecal incontinence that is effective when others fail, and may therefore be of benefit.

Methods: Five women, median age 61 (30–71) with faecal incontinence secondary to scleroderma underwent sacral nerve stimulation. Median preoperative weekly incontinent episodes was 15 (7–25), duration of incontinence was 5 years (5–9) and of scleroderma 13 years (4–29). All had failed maximal treatment including behavioural therapy. Bowel diary, quality-of-life assessment (SF-36), endoanal ultrasound and anorectal physiology were performed before and after treatment.

Results: At median follow-up of 26 months (8–62), four patients were continent, one failed temporary stimulation. Median weekly incontinent episodes decreased (15, 11, 23, 7 *versus* 0, 0, 0, 0). Urgency resolved (median ability to defer < 1 min *versus* 12.5 min [5–15]). Quality-of-life scores showed an overall improvement. The internal anal sphincter was atrophic, median width 1.0 mm (0–1.6, normal range 2.4–3.4 mm). Anorectal physiology revealed an increased maximal anal resting pressure (37 [10] cm H20 (median [standard deviation]) *versus* 65 [16]) and squeeze pressure (89 [48] *versus* 105 [67]). Rectal sensation improved at threshold (53 [17] ml air *versus* 33 [20]), urge (83 [18] *versus* 58 [25]) and maximum tolerated volume (143 [23] *versus* 75 [34]). There were no major complications.

Conclusions: Sacral nerve stimulation is a safe and effective technique for the treatment of faecal incontinence in patients with scleroderma when other treatments have failed.

Poster 02

The surgical treatment of idiopathic constipation with sacral nerve neuromodulation

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Aims: Severe constipation usually responds to conservative therapies, however, a small proportion of patients require more intensive treatment, and surgery provides variable results. Previously unreported, permanent sacral nerve stimulation may be of benefit in these patients.

Methods: Four women, aged 27–36 years underwent temporary, and then permanent sacral nerve stimulation. All had severe constipation, resistant to maximal treatment, including behavioural therapy. Preoperative duration of symptoms was 8–32 years. Gut transit time was prolonged in two patients. Clinical evaluation, 3-week bowel diary, Wexner score, symptom analogue score, the SF-36 quality of life score and anorectal physiology were performed before and after treatment.

Results: There was a marked symptom improvement in all patients with temporary, and in three with permanent stimulation. Median follow-up was 12 months (5–15). Bowel frequency improved (1, 6, 1 and 5 evacuations/3-weeks *versus* 21, 28, 17 and 6). There was an improvement in evacuation score ([0–4] 0, 4, 4, 4, *versus* 0, 1, 1, 4), the per cent time with abdominal pain and bloating (95, 100, 95, 100 per cent *versus* 0, 10, 14, 100 per cent), the Wexner score ([0–30] 22, 20, 22, 20 *versus* 1, 9, 9, 20), the analogue score ([0–100] 38, 28, 16, 17 *versus* 98, 75, 84, 20) and quality of life. Anorectal physiology demonstrated increased maximal anal resting and squeeze pressures. Rectal sensation to distension and electrostimulation improved. Transit time normalized in one patient and there were no major complications.

Conclusions: Permanent sacral nerve stimulation can effectively treat patients with idiopathic constipation when other therapies have failed.

Poster 03

Combination treatment of colorectal liver metastases with resection and radiofrequency ablation

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Aims: Radiofrequency (RF) ablation is increasingly accepted as a treatment option in patients with unresectable colorectal liver metastases. In this study, we assess outcome in patients treated with a combination of liver resection and RF.

Methods: Sixteen patients with multiple or unresectable colorectal liver metastases were treated. Six had RF at open laparotomy, three had synchronous liver resection and RF, seven had metachronous liver resection and percutaneous RF. A total of 27 tumours, diameter 1.2–10 cm were treated with high-power, internally water-cooled RF using single or cluster electrodes. Intraoperative US was used to guide electrode placement and monitor the bubble cloud. All patients were followed with serial CT scans.

Results: Eleven are alive at a median follow-up of 21 months (2–97) from the diagnosis of liver metastases. Seven of 11 (64 per cent) have no evidence of residual or recurrent liver tumour on contrast-enhanced multislice CT. Five patients have died at 12, 23, 33, 40 and 67 months, respectively. There were three complications, a pleural effusion requiring percutaneous drainage, focal grounding pad burns in a cachectic patient with bilateral hip replacements and severe transient hip pain 18 h postprocedure which resolved spontaneously. Two of six (33 per cent) intraoperative ablations were incomplete reflecting the difficulty of using US to monitor treatment efficacy in all but small (<2 cm) lesions.

Conclusions: Intraoperative radiofrequency ablation in combination with resection can be used to extend treatment to patients previously considered nonsurgical candidates.

Poster 04

Improvements in surgical care for patients with diverticular disease: evidence from 799 deaths peer-reviewed for the Scottish audit of surgical mortality

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Aim: To seek evidence of improvements in surgical care of patients with diverticular disease.

Methods: From 1994 to 1999, 799 patients died with a principal diagnosis of diverticular disease. As part of the Scottish Audit of Surgical Mortality (SASM), each case was peer reviewed by a surgical assessor and, for the 396/799 (50 per cent) who had surgery, by an additional anaesthetic assessor.

Results: In the first 3 years, the common causes of death were perforated diverticulitis, septicaemia, bronchopneumonia and cardiac events. Multiple organ failure and pulmonary embolism became more frequent as the cited cause of death by 1999. Adverse factors contributing to death fell from 22/135 (16 per cent) in 1994 to 8/153 (5 per cent) in 1999. The common adverse factors were delay in transfer to surgical care or delay to operation; a missed diagnosis or wrong operation performed became less common. For 10/799 (< 1 per cent) patients (nine operative cases), adverse factor(s) caused death; these were complications of surgery and delays in management. In two patients operated

upon, anaesthetic care caused death. For patients who were graded ASA 3, 4 or 5 at the time of surgery, senior surgeon and anaesthetist input rose over 6 years. For emergency admissions during 1997–1999, the timing of surgery was recorded: by 1999 fewer patients who subsequently died had their operation between 18.00 hours and 24.00 hours.
Conclusions: SASM has mapped a reduction in adverse clinical decisions contributing to death from diverticular disease. However, inflammatory and cardiovascular complications of the disease process remain a significant challenge.

Poster 05

Acquired coagulation Factor XIII deficiency following colonic surgery

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Aims: To assess the effect of colorectal excision surgery on Factor XIII (FXIII) levels, and to establish any correlation between acquired FXIII deficiency and outcome.
Methods: Fifty-two patients undergoing elective laparotomy and bowel resection for colorectal malignancy were recruited to the study. Venous blood samples were collected preoperatively, and on the first, third and seventh postoperative days. FXIII levels were calculated against known plasma standard. A record was made of any postoperative complications.
Results: The mean FXIII level preoperatively was 145 per cent of normal. One patient was FXIII deficient preoperatively (<70 per cent normal). Day 1 postoperative mean FXIII level was 110 per cent with six patients < 70 per cent. Day 3 mean FXIII was 97 per cent with nine patients < 70 per cent. Day 7 mean FXIII was 103 per cent again nine patients < 70 per cent. Thirteen of the 51 (25 per cent) patients with normal preoperative FXIII levels became FXIII deficient in the postoperative phase, with the average fall in FXIII between preoperative levels and day 3 postoperative being 48 per cent. Fifteen of the 52 patients had a recorded complication; this group of patients had a significantly larger drop in FXIII levels than those without a complication (median 51 per cent *versus* 23 per cent, Mann–Whitney $P = 0.009$).
Discussion: It would appear that the surgical insult caused by colorectal excision is sufficient to cause acquired FXIII deficiency, and this may be associated with postoperative complications. Further work is ongoing, but if the larger series confirm these results, FXIII substitution therapy in those patients who have significant falls in FXIII levels could potentially reduce postoperative complications.

Poster 06

Nonspecific colonic ulceration in patients with chronic renal failure

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Aim: Nonspecific colonic ulceration (NSCU) is a rare complication of patients with chronic renal failure (CRF). Our aim was to review the presentation, management and histopathological features of patients with NSCU.
Methods: We searched the histopathology database for colon and rectum samples from patients under the care of renal physicians from 1994 to 1999. Samples were then reviewed by a consultant histopathologist in conjunction with the patient notes.
Results: We identified 14 patients with a diagnosis of NSCU. On reviewing the histology, four had pathological features suggestive of a specific diagnosis, including one with cytomegalovirus. Six of the 10 patients remaining had polarizing crystals in the submucosa or mucosa, suggesting oxalate deposition. The patients had a mean age of 59 years (SD ± 10), and had required renal replacement therapy for between 12 and 192 months. Patients presented with either abdominal pain (six) or lower gastrointestinal bleeding (four). The site of the ulcers varied from right colon (four), transverse colon (two), splenic flexure (one) and left colon (three). Six required a colonic resection (right hemicolectomy: four; transverse colectomy: one; subtotal colectomy: one). Four of these patients presented with generalized peritonitis due to colonic perforation. Of the four patients with bright red rectal bleeding, one was found to have ulcers in the caecum.
Conclusion: Patients with CRF presenting with abdominal pain or rectal bleeding should undergo full examination of the colon. NSCU is a cause of colonic perforation and surgeons should consider this in CRF patients presenting with an acute abdomen.

Poster 07

Shorter hospital stay associated with fast track postoperative care pathways and laparoscopic intestinal resection are not associated with increased physical activity

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Aims: Recent prospective studies have shown that 'fast-track' postoperative care protocols (FT) can reduce hospitalization after major intestinal surgery to 4.5 days, as compared to the 7–10 days with traditional management (TR) and 2.5 days after laparoscopic surgery (LC). We used computerized actigraphy (CA) to evaluate physical activity using TR, FT and LC approaches.
Methods: Fifteen patients undergoing intestinal resection by LC, TR and FT were recruited. CA devices were placed on the wrist and ankle of each patient from day 1 until hospital discharge. Intrapatient and intergroup analyses were performed using 24 h evaluations from 08.00 hours to 16.00 hours. Results are presented for level of activity (LOA; activity counts per minute), activity index (AI; per cent epochs with activity) and acceleration index (ACI; change in activity rate during analysis period) for 8.00–16.00 hours.

	Wrist LOA	Ankle LOA
TR	6263 ± 8008	1640 ± 3795
FT	7874 ± 8550	2153 ± 4838
LC	8526 ± 9917	2326 ± 4905

Results: LOS was significantly shorter in LC than FT and TR patients ($P < 0.01$). There was no significant increase in wrist LOA for FT or LC patients. Ankle LOA, evaluated to determine whether this site was more accurate, showed no difference between groups. Similarly, there was no difference in AI or ACI.
Conclusions: Although CA is an easily standardized method of evaluating physical activity after major abdominal surgery, no difference can be demonstrated between activity levels in patients managed by differing care pathways. Reductions in stay may be associated with factors other than a change in the level of physical activity after surgery.

Poster 08

Specialist colorectal nurse-run anal fissure clinic

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Aims: Specialist colorectal nurse practitioners are knowledgeable and experienced in areas ranging from stoma care to endoscopy, and are invaluable to their departments and patients in both the hospital and the community. We report the experience of the anal fissure/GTN clinic run by our specialist colorectal nurse.
Methods: After initial consultation with the consultant colorectal surgeon, all patients with anal fissures suitable for GTN ointment treatment were referred to the colorectal nurse who would commence treatment. She was to be contacted by phone, to provide advice, or a further consultation. The patients were routinely followed up 3, 6, and 12 weeks after starting treatment. Patient information was prospectively collected.
Results: Thirty-seven patients have been seen to date. Twenty-three patients (62 per cent) were cured; of these, there were three recurrences (8 per cent) which all healed with a repeat course of treatment. Seven patients (19 per cent) were considered failures and had surgery. Two patients were noncompliant

due to intolerable side-effects. Seven patients (19 per cent) had their symptoms relieved, but the fissures were not clinically healed. The patients who only had symptom relief (19 per cent) or had recurrences (8 per cent) would normally have proceeded to surgery. In the current clinic, it was possible to avoid surgery, because the patients were given more intensive follow-up and further GTN treatment when necessary, all within days of the symptoms returning.

Conclusions: Our anal fissure clinic run by a specialist colorectal nurse has significantly better clinical outcome and patient satisfaction than previous reports, and is therefore superior to treatment and follow-up by the routine clinics or the GP.

Poster 09

Limitations in the investigation of the anorectum

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Aims: Anorectal investigations are an important part of the assessment of the anorectum. New catheter designs, improved software and transanal ultrasonography equipment allow for a more detailed assessment of the anorectum. The aim of this study was to determine the degree to which anorectal investigations explained patients' symptoms.

Methods: Data was collected prospectively from all patients who underwent anorectal investigation over an 18-month period. Assessment included a clinical history, anorectal manometry, anal electrosensitivity and endoanal ultrasonography.

Results: A total of 361 consecutive patients were included: 273 females of median age 50 (IQR 39–64) years and 88 males of median age 50 (IQR 34–66) years. Patients had symptoms of faecal incontinence in 167 (46 per cent), constipation or obstructive defaecation in 75 (21 per cent), leakage in 47 (13 per cent), urgency in 14 (4 per cent) and miscellaneous in 58 (16 per cent). Of the 74 patients with a sphincter defect demonstrated by ultrasound, 43 (58 per cent) had normal anal canal pressures. Of the 75 patients with constipation who were continent, 28 (37 per cent) had low anal canal pressures, neuropathy or a sphincter defect. Of the 167 patients with faecal incontinence, 33 (20 per cent) patients had normal investigations.

Conclusions: The majority of patients with sphincter defects have normal pressures. Over one-third of patients who are continent have abnormalities that suggest a deficiency of the sphincter mechanism and one-fifth of patients with faecal incontinence have normal investigations. Patients' symptoms are only partly explained by anorectal investigations. New techniques such as resting pressure gradient calculation and transperineal ultrasonography may provide a more comprehensive assessment.

Poster 10

Functional outcome of rectopexy for obstructive defecation – what do the patients think?

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Aims: Obstructive defecation (OD) is characterized by prolonged straining at stools and a sense of incomplete emptying. This may be secondary to rectal intussusception, and rectopexy has been used to correct this abnormality and thereby improve OD.

Method: A cohort of patients who underwent rectopexy for OD between January 1997 and December 1998 was identified from the computer records and their case notes retrieved and analysed. Thirty-six patients (M:2; F:34) (median age: 54 years; range: 20–71) underwent surgery for OD. A simple functional assessment questionnaire was sent to 35 patients by post. The identity of the patient was kept anonymous. Twenty-seven patients (77 per cent) responded.

Results: Following surgery, sustained improvement in OD was noted by 28 per cent, while 18 per cent felt an initial improvement only. No change was reported by 30 per cent, while 24 per cent felt they were worse. Ten patients (37 per cent) had faecal leakage which resolved in two (20 per cent), improved in five (50 per cent) and persisted in two (20 per cent). Prior to surgery, 14 (52 per cent) were digitating rectally to aid defaecation. This resolved in one (7 per cent), improved in four (29 per cent), while six patients (43 per cent) felt no change and three (21 per cent) felt worse. Following surgery, laxative usage increased from 58 to 78 per cent. Ten patients (37 per cent) were satisfied with the outcome of surgery. Five patients (19 per cent) would recommend a rectopexy to someone with similar symptoms.

Conclusion: The overall patient satisfaction following rectopexy for OD is less than ideal. Hence careful patient selection and counselling prior to surgery is essential.

Poster 11

Outcome of long-course radiotherapy for rectal cancer

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Aims: The use of preoperative radiotherapy in the treatment of locally advanced rectal cancers is established as a technique. We prospectively audited the outcomes of all referrals to one regional oncology unit.

Methods: All patients with locally advanced rectal cancer referred to the oncology unit for long-course radiotherapy from February 1997 to January 2001 were included. This included referrals from seven regional hospitals.

Results: There were 48 patients who underwent long-course radiotherapy (13 female) with a mean age of 64 years (range: 38–81). All received 45 Gy in 25 fractions of four field pelvic radiotherapy over 6 weeks. The median time from referral to radiotherapy was 34 days (range: 18–83). All tumours were fixed at preoperative EUA assessment. After radiotherapy, nine were assessed as still fixed, and 30 were documented as regressing or smaller. The overall 1-year survival rate was 50 per cent, with a median survival of 9 months (range: 1–47). Twenty-nine (58 per cent) patients went on to have a resection, and the median survival was 12 months (range: 3–47) (significantly different $P < 0.01$, Mann–Whitney *U*-test). There were four significant postoperative complications (fistula, MI, bleeding, stomal prolapse).

Conclusions: Patients with locally advanced rectal cancer have a poor overall prognosis. Long-course radiotherapy downstages some tumours, and resection offers a small survival benefit. The survival benefit is however small, and the quality of life for the patient should be considered as most important when discussing surgery.

Poster 12

Laparoscopic surgery for Crohn's disease: 10-year experience

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Aims: Even though laparoscopic surgery is an established technique for the management of complicated Crohn's disease, there are very few series reported from the UK.

Methods: Data was gathered from the case notes of patients who had undergone laparoscopic procedures for Crohn's disease in the period between 1991 and 2001.

Results: Fifty-seven laparoscopic procedures were performed on 48 patients. There were 17 males and 31 females. The mean age was 35.7 years (SD = 13.2). Indications for surgical intervention were active disease in 19 cases, strictures in 23, internal fistulae in 6 and other reasons in 9. Thirty-one were primary procedures and 26 were procedures for recurrence. Twenty-eight of these procedures were ileo-colic resections, 11 were small bowel procedures, 2 were subtotal colectomies, 3 were left hemi-colectomies and there were 13 other procedures performed (adhesiolysis, ileostomy and stapled fistulectomy). The mean blood loss was 340.5 (SD = 389.2) cm^3. The mean operative time was 130.4 (SD = 45.1) min. The median time to return to a solid diet was 5 days (range: 2–8). The median hospital stay was 8 days (range: 4–47). The conversion rate was 28 per cent. The major complication rate was 15.8 per cent (9 of 57). There were six other cases of minor complications. The recurrence rate was 24.5 per cent. The mean follow-up was 43 (SD = 26) months.

Conclusions: Laparoscopic surgery for Crohn's disease is safe with a low complication rate. Seventy-two per cent of patients undergo laparoscopic surgery successfully and enjoy the benefits of an early return to normal diet and a shorter hospital stay.

Poster 13

Does chromendoscopy have a role in the detection of dysplastic colonic epithelium?

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Aims: Early detection and treatment of colonic cancer improves survival but endoscopic detection of dysplastic epithelium maybe difficult. This study was designed to assess the usefulness of vitalstaining in detecting abnormal epithelium.

Methods: Patients eligible for the study comprised a high-risk group for colonic cancer (polyps, ulcerative colitis, previous colonic cancer, family history). Bowel preparation was with picolax or phosphate enema. Areas of colonic or rectal mucosa which appeared abnormal were sprayed with toludine blue solution through an endoscopic cannula and left *in situ* for 1 min before being washed with 1 per cent acetic acid solution. Areas taken up by the dye were biopsied (dye positive). Suspicious areas not taken up by dye were also biopsied (dye negative). All endoscopic procedures were by the same surgeon and histology reporting by single pathologist.

Results: Over a 10-month period 30 patients underwent chromendoscopy, age range 52–88 (mean 68.4; median 7) years. Vital staining was positive in 25 patients. Nine showed dysplastic epithelium, 11 inflammatory changes and five normal mucosa. Five biopsies were dye negative (four metaplastic polyps, one malignant polyp). Sensitivity for identification of dysplastic or malignant epithelium was 90 per cent and specificity 20 per cent. Negative predictive value was 80 per cent and positive predictive value 36 per cent.

Conclusion: The study has shown that vitalstaining with toludine blue is a useful adjunct to endoscopy and detects dysplastic epithelium. Although the test has a high sensitivity, it lacks specificity and does not differentiate between dysplasia and inflammation.

Poster 14

Virtual colonoscopy in colorectal surgery

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Aims: Virtual colonoscopy (VC) involves interactive real-time manipulation of helical computer tomography data to produce two- and three-dimensional images of the entire colon, permitting endoluminal fly through as well as surface and volume rendering. It is minimally invasive; it may be safer, more cost effective, and better accepted by patients than colonoscopy. This pilot study evaluates the accuracy of VC with colonoscopy/barium enema (C/BE) as a reference standard.

Methods: Forty-seven patients (25 male; mean age: 72 years; range: 40–92) presenting to the colorectal clinic underwent VC with a back-to-back C/BE. Data acquisition and analysis was performed using Easy Vision Endoview 3-D software. The results of the VC were compared with the findings of conventional C/BE and correlated with outcome. In 11 of the 47 patients who had abnormal BE/C, the findings on VC were all confirmed at surgery. Nodal status concurred at surgery. Twenty-nine of the 47 patients with abnormal or equivocal BE/C had a normal VC and were discharged from follow-up.

Conclusions: Virtual technology has the advantage of detecting abnormalities proximal to obstructing carcinomas, accurate localization of abnormalities within the colon and good patient tolerance. The sensitivity of VC appears to be remarkably high. VC may play an important role in future colorectal surgery.

Poster 15

Missing colorectal cancer – the 2-week wait

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Aims: The NHS Cancer Plan aims to improve patient confidence in the cancer care system, and states that as soon as cancer is suspected, the patient must be seen within 2 weeks of receipt of an urgent referral. This study assesses the introduction of guidelines and an urgent referral proforma for colorectal cancer at our hospital, following the national implementation of the 2-week standard in July 2000.

Methods: Prospective data was collected for all referrals for an 8-month period commencing July 2000 (353 cases). There were 152 (43 per cent) general practitioner-initiated proforma, 89 (25 per cent) consultant surgeon-initiated proforma and 112 (32 per cent) routine referrals.

Results: Eighty-four per cent of referrals described symptoms meeting the proforma guidelines. Only 46.6 per cent of these were referred by general practitioners. Analysis of all cases meeting the criteria showed the proforma to have a sensitivity of 95 per cent, and a negative predictive value of 96 per cent for colorectal neoplasia (cancer and polyps) ($P = 0.03$). When applied to the actual proforma referrals received the proforma was not predictive for neoplasia (ns). Analysis of individual proforma criteria shows none of these to be independently predictive for colorectal neoplasia. A total of 97.5 per cent of proforma referrals were seen within 2 weeks.

Conclusion: We have shown that with 100 per cent uptake of the proforma system, 5 per cent of cancer cases will be missed. The only way to give cancer patients guaranteed access to optimal cancer care is for all colorectal referrals, irrespective of symptoms, to be seen within 2 weeks of receipt of a referral.

Poster 16

The impact of microscopic extranodal tumour deposits on the outcome of patients with rectal cancer

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Aims: Microscopic mesorectal soft tissue extranodal deposits (END) discontinuous with the primary tumour are identified in many rectal adenocarcinomas. We have studied the impact of these deposits on the outcome of patients with rectal cancer.

Methods: Histology slides were reviewed from 84 patients whose resection specimens for rectal cancer were staged as Dukes' C, or Dukes' B with ENDs. Fourty-four patients had ENDs (30 M and 14 F), and 40 controls had lymph node involvement only (25 M and 15 F). Outcome was assessed in terms of local and systemic control and survival.

Results: Distant metastases were diagnosed earlier in patients with ENDs (mean: 14 months), compared to controls (mean: 37 months) ($P = 0.001$). Twenty-five per cent of the END-group patients, but only 7.5 per cent of the controls developed hepatic metastases ($P = 0.02$). Local recurrence was seen in 13.6 per cent patients of the END group and 7.5 per cent of the controls ($P = 0.47$). Cancer-related mortality was higher in the END group (20 patients *versus* 10 patients, $P = 0.025$). The 3-year actuarial survival was 43.18 per cent in patients with ENDs and 67.5 per cent in those without. A significant association was noted between the number of ENDs and intramural vascular invasion ($P = 0.002$), perineural invasion ($P = 0.025$) and lymph node involvement ($P = 0.01$). There was no similar correlation with extramural vascular invasion.

Conclusion: These data suggest that END is a distinct form of metastatic disease in patients with rectal cancer. These tumour foci should be considered as indicators of poor prognosis.

Poster 17

The role of CT colonography in colonic strictures

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Aims: Strictures of the colon by diverticulosis or carcinoma prevents visualization of the lesion and the proximal colon by barium study or colonoscopy which is particularly essential in carcinoma to rule out a synchronous lesion. This study examines CT colonography (CTC) as an alternative imaging modality.

Methods: Thirty-three patients (20 men; median age: 69 years) with colonic strictures on colonoscopy underwent CTC following standard bowel preparation, intravenous contrast and rectal air insufflation. The CTC and colonoscopy findings were compared.

Results: Colonoscopy suspected carcinoma in all 33 patients of which two were associated with diverticular strictures. Colonoscopy failed to visualize the proximal colon in all patients. CTC showed appearances of carcinomatous stricture in 31 patients and of diverticulosis in two. The resected specimens confirmed colonic carcinoma in 30 patients and diverticular stricture in three. The proximal colon was adequately outlined by CTC in all patients. CTC identified a synchronous carcinoma in one and tubulovillous adenoma in two other patients that were confirmed intraoperatively and resected.

Conclusions: CTC should be considered as an alternative imaging technique to evaluate the lesion and proximal colon in patients with colonic strictures. However, CTC may not be able to clearly distinguish carcinomatous change in diverticular strictures in some cases.

Poster 18

A new method of assessing anal sphincter integrity using inverted vectormanometry

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Aims: Vectorgraphy, with integrated mapping of radial pressure profiles of the anal canal, has doubtful reliability in identifying pressure-related defects, and poor correlation with endoanal ultrasound (EAUS) scans. We aimed to devise a technique to enable vectorgraphy to be more representative of sphincter function and integrity and be of clinical guidance.

Methods: Vectormanometry was performed in 50 patients with anorectal disorders using a standard Arndorfer pneumo-hydraulic system. Three-dimensional manometric images of each 0.5-cm 'slice' of the anal sphincter were computer generated by plotting anal pressures at rest and during squeeze in all the eight channels at each station, radially around a central zero axis. The graphs were then inverted with zero at the periphery and the maximal anal pressure at the centre. Both 'inverted' and standard vectorgraphs were compared with EAUS images at similar levels, assessing internal and external anal sphincters.

Results: Standard vectormanometry produced excellent profiles of pressures throughout the anal canal; however, the anatomical interpretation of the anal canal bore no resemblance to the pictures produced by EAUS. When 'inverted' vectorgraphy was used, the graphs generated showed a much better correlation with EAUS pictures at each 0.5-mm level of the anal canal. This was true both for squeeze pressure graphs and external sphincter correlations, as well as for resting pressure graphs and internal sphincter correlations.

Conclusions: Accurate assessment of sphincter integrity is difficult when interpreting the vectormanometry graphs in the current format. 'Inverted' vectorgraphy gives good correlations with EAUS and provides combined functional (pressure measurement) and anatomical (three-dimensional profile) information regarding the anal canal.

Poster 19

The 2-week colorectal cancer referral pathway – is it effective?

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Aims: NHS Executive guidelines for the referral of patients with suspected colorectal cancer (CRC) were introduced in an attempt to reduce the time to diagnosis and treatment. The aim of this study is to assess whether the 2-week referral pathway has achieved a reduction in the wait to first outpatient appointment (OPA), diagnosis and treatment.

Methods: Data were collected on all CRC patients seen in the specialist clinic via the fast-track referral system between March 2000 and December 2001. Days waited from referral to OPA, days to diagnosis from OPA and days to first treatment from date of diagnosis were calculated. This data was compared to that calculated for CRCs referred in the same time period via GP letter and a historical control group of all CRC diagnosed between March 1998 and December 1999.

Results: Between March 2000 and December 2001, 137 patients with CRC were seen, 51 (37 per cent) as a result of fast-track referrals and 86 (62 per cent) referred via GP letter. In the historic control group, there were 136 patients with CRC. The table demonstrates the delay to OPA, diagnosis and treatment for the three groups.

	Fast-track referrals	GP letter referrals	Historic controls
Days waited (referral to OPA)	10.9	26.2	10.2
Days to diagnosis (OPA to diagnosis)	7.8	16.7	8.6
Days to first treatment (diagnosis to treatment)	12.6	8.6	6.8

Conclusions: The 2-week wait referral system has failed to reduce the time to diagnosis and treatment of colorectal cancer. The majority of CRC patients are currently not referred as fast tracks (63 per cent), and in these patients the time to diagnosis has in fact increased.

Poster 20

Peritoneal adhesion formation after laparotomy may be accentuated by postoperative bacterial translocation

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Aims: As translocation of enteral bacteria and their peptides potently activates immune responses to surgery, we assessed and modulated the effects such peritoneal contamination has on adhesion formation after surgery.

Methods: Two endotoxin-hyposensitive mouse strains (C3H/HeJ and C57/ScCr) along with their synergic counterparts (C3H/HeN and C57/ScSn) underwent reproducible adhesion-inducing surgery (AIS) ($n = 10$ per group) with blinded grading of adhesions (0–3) 14 days later. To reflect the time course of bacterial translocation, CD-1 mice underwent either AIS or sham laparotomy 1 h after gavage with FITC-LPS ($5\,\mathrm{mg\,mL^{-1}}$), their peritoneal macrophages being assessed 2, 6 and 10 h later by flow cytometry. Additional CD-1 mice had AIS, received either bactericidal/permeability-increasing protein (BPI, 2 mg per mouse) or vehicle solution intraperitoneally at 0, 2, 4 and 6 h postoperatively

Results: Both HeJ and ScCr mice had significantly less adhesions than their respective controls FITC-LPS in peritoneal macrophages was greater in the AIS group for 6 h postoperatively. Postoperative BPI markedly attenuated subsequent adhesion formation (see Table)

HeJ/HeN	1 4 (±0 5)*/2 6 (±0 6)
ScCr/ScSn	1 2 (±0 4)*/2 7 (±1 0)
BPI/vehicle	0 8 (±0 2)*/1 7 (±0 2)

Mean channel fluorescence of peritoneal macrophages (data are mean ± SE)

Time after surgery (h)	2	6	10
Adhesion-inducing surgery (AIS)	121 ± 6*	27 ± 1*	0
Sham laparotomy (Sham lap)	43 ± 1	15 ± 2	0

Mean (±*SE*) adhesion scores for each group/control ($^{*}P < 0\ 05$, Mann–Whitney *U*-test/ANOVA)

Conclusion: Peritoneal adhesion formation after laparotomy may be exacerbated by perioperative bacterial translocation, an effect markedly diminished by appropriately administered perioperative antibacterial agents

Breast

Poster 21

The expression of the breast cancer marker mammaglobin in peripheral blood and bone marrow

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Aims: Mammaglobin is expressed in over 80 per cent of breast cancers, and though it is not expressed in healthy axillary nodes, it has been detected in those containing metastases and it has also been shown to improve the detection of micrometastases in axillary nodes. Patients with tumours that express mammaglobin may also have expression in peripheral blood and bone marrow, and we therefore wish to identify those patients with mammaglobin expression detectable in their tumours and the value of mammaglobin as a marker in bone marrow and peripheral blood.

Methods: The tumour, axillary nodes (including sentinel nodes), peripheral venous blood and sternal bone marrow were assayed for mammaglobin expression using a nested reverse transcriptase polymerase chain reaction

Results: In 72 consecutive patients with proven breast cancer, mammaglobin was expressed in 18 patients' bone marrow and 16 patients' peripheral blood Of the 20 patients where mammaglobin was expressed in lymph nodes, nine had expression in the bone marrow and six in peripheral blood. Of the 17 patients in whom the tumour showed lymphovascular invasion, four had expression in blood and six in the bone marrow

Conclusion: Mammaglobin has been shown to be a reliable marker in lymph nodes in patients with breast cancer indicating metastatic disease This marker has also been identified in peripheral blood and bone marrow, continuing follow-up of these patients will determine its value and prognostic significance.

Poster 22

Quality assurance audit in axillary surgery

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Aims: The results of Breast Test Wales (BTW) audit conducted in 1998 and circulated to the consultants involved in 2001 has shown that sampling/clearance of more than four lymph nodes was not achieved in four out of the 17 trusts in Wales, of which ours was one This study aimed to assess the accuracy of BTW audit in our firm and to find out if our results were in keeping with the BASO guidelines.

Methods: The records of all patients operated for symptomatic breast cancers during the calendar year 1998 were retrospectively reviewed.

Results: Some 82 patients underwent surgery for invasive breast cancer from January to December 1998. Forty-nine (60 per cent) patients underwent axillary sampling, whilst 33 (40 per cent) underwent axillary clearance. Only 65 patients (79 per cent) underwent sampling/clearance of more than four lymph nodes (BASO standard: >90 per cent) We were disappointed to find that the results were in keeping with BTW audit and that our current practice of axillary sampling fell short of the BASO guidelines.

Conclusions: It has been our surgical policy to remove fat and lymph nodes en block from level 1 region and rely on the pathologist to find lymph nodes from this block of tissue. We hope that by changing to a more secure policy, i e dissecting out at least four lymph nodes from the axilla and presenting them to the pathologist, we would be able to achieve the quality outcome measure in axillary surgery

Poster 23

Pathological complete response and improved breast conservation rates after primary medical therapy for operable early breast cancer

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Aims: A number of studies have shown that primary medical therapy (PMT) achieves clinical overall response (cOR) rates of 70–90 per cent in women with operable early breast cancer However, pathological complete response (pCR) is achieved in <20 per cent of cases

Methods: We reviewed response rates to PMT in 29 women, aged 31–76, with stage T2–T4 breast cancer treated with various chemotherapeutic regimens between 1997 and 2001

Results:

1 Twenty-three of 29 (79 per cent) patients exhibited cOR and 13/29 (57 per cent) exhibited clinical complete response (cCR). Three patients with cCR had recurrence within 19 months of treatment
2 Eight of 22 (36 per cent) patients proceeding to surgery exhibited pCR. There has been no recurrence in this group so far.
3 Of the 18 patients planned for mastectomy at presentation, 7 (39 per cent) were suitable for breast conservation after PMT

Conclusion: As pCR may be associated with improved overall survival, our results are encouraging: PMT can increase breast conservation rates and allows evaluation of response to chemotherapy PMT should be considered as an alternative to conventional adjuvant therapy in operable early breast cancer.

Poster 24

Needle biopsy in the breast clinic: core biopsy, fine-needle aspiration or both?

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Aims: To assess whether the use of the combination of core biopsy (CB) and fine-needle aspiration cytology (FNAC) is better than either alone in the diagnosis of palpable breast cancer.

Methods: The policy of our breast unit is to perform both CB and FNAC on all symptomatic lumps A member of the clinical team (not a radiologist or pathologist) performs these without imaging. The histology of all symptomatic patients presenting in the last 2 years was reviewed The sensitivities of CB, FNAC and the combination of both were recorded Tumour parameters were also examined.

Results: A total of 246 symptomatic breast cancer patients with a median age of 61 presented over this period The sensitivity of FNAC was 75 per cent and of core biopsy 85 per cent When both investigations are used in conjunction, the sensitivity was 91 per cent The sensitivity improved with increasing lump size.

Conclusion: FNAC and CB have a reasonable sensitivity, but the combination of both is superior. We recommend the use of both FNAC and CB for the assessment of symptomatic breast lumps in a district hospital breast clinic.

Poster 25

Does pregnancy termination affect subsequent breast disease?

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Aims: Controversy exists whether abortion increases the risk of subsequent breast cancer, though many studies imply this association.
Methods: We surveyed the new female patients attending a one-stop breast clinic from December 1999 to February 2000 inclusively. In addition to the usual demographic and medical data, 387 new patients were asked about their total number of pregnancies and births to living children.
Results: Of these women, 36 were diagnosed to have a breast cancer and 351 to have benign breast disease: 10.25 per cent (4 women out of 36) of the breast cancer patients and 29.6 per cent (104 out of 351) of the benign breast disease patients ($P < 0.01$) had had an abortion. During the same period, the therapeutic abortion rate for the general population was calculated at 2.2 per cent for 16–35-year-old females ($P < 0.001$). There were no significant differences between the number of pregnancies or the number of living children between the two groups (0–5 *versus* 0–12, $P = 0.62$ and 0–5 *versus* 0–7, $P = 0.68$).
Conclusions: Sudden interruption of a pregnancy may induce dramatic hormonal changes in the breast, making subsequent breast disease more likely. This favours a more benign than malignant correlation.

Poster 26

The '2-week rule' referral – is age of patient a safe criteria?

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Aims: Government directive requires that patients referred by general practitioners with suspected breast cancer be seen within 2 weeks. The aim of this study was to see if 'age of patient' would be a safe criterion to assess suitability for such a referral.
Methods: All '2-week rule referrals' to the breast unit for a period of 6 months (February 2001–July 2001) were assessed for age of referral, symptoms of referral and number of confirmed cancers. This was compared with the 'non 2-week rule' referrals received during the same period.
Results: Of the total 1435 referrals in the above period, 335 patients (23 per cent) were referred under the 2-week rule. Of these, 61 patients (18 per cent) were confirmed having breast cancer. Eighty-five of the patients (25 per cent) were under the age of 40 of which two had breast cancer (2 per cent). Of the 61 breast cancer patients, 57 (93 per cent) presented with a palpable breast lump and only one patient presented with the symptom of breast pain. Of the 1100 cases referred under the 'non 2-week rule' criteria, 36 (3 per cent) patients had breast cancer.
Conclusions: In our experience, during the study period prioritization by general practitioner was safe. Patients under the age of 40 with no clinically palpable lump can be safely referred as routine, this may reduce the pressure created by the '2-week rule'.

Upper Gastrointestinal

Poster 27

Staging of gastric cancer in the West Midlands – appraisal of compliance with the UICC/AJCC staging system in 1644 cases

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The fifth edition of the UICC TNM staging system for gastric cancer has been shown to be of greater prognostic importance than the Japanese system and suggests that the number of metastatic lymph nodes may be of greater prognostic value than their location. For accurate staging however, this system requires at least 15 lymph nodes to be identified in the resected specimen.
Aims: To assess the accuracy of gastric cancer staging by applying the latest TNM criteria to all resected cases in the West Midlands from 1998 to 1999.
Methods: Data was obtained from the West Midlands Cancer Intelligence Unit for all cases of gastric cancer registered from 1998 to 1999. The number of lymph nodes detected in all resected specimens was recorded in order to assess the proportion of cases in which TNM staging was feasible.
Results: A total of 1644 cases of gastric cancer were registered from 18 units in the region, with 699 (42 per cent) undergoing attempted curative resection. Fifteen or more lymph nodes were retrieved in 217 (31 per cent) operative cases, with a range of 11–76 per cent across units.
Conclusions: A multidisciplinary approach is critical to the effective management of gastric cancer. This study highlights the need for close collaboration between surgeons and pathologists in order to improve the accuracy of gastric cancer staging.

Poster 28

Factors predicting common bile duct stones (CBDS) in patients with gallstone disease: the value of magnetic resonance cholangiopancreatography (MRCP)

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Aim: Even with the application of available predictive scoring systems for detection of CBDS, a large number of normal ERCPs are performed, with subsequent morbidity. The aim of the present study was to create a predictive model for CBDS and to assess the value of MRCP in order to detect CBDS in a consecutive series of unselected patients with gallstone disease planned for cholecystectomy.
Methods: A total of 366 patients with gallstone disease (M/F = 118/248; mean age: 57 years; range: 8–84 years) underwent cholecystectomy in our unit during the year 1998. Statistical analysis was performed on patient data obtained at the time of first presentation to the hospital. Simple logistic regression was used to explore the relations of different variables with demonstrable CBDS. To create a predictive model for CBDS, multivariate analysis was done, and the value of MRCP was assessed in this predictive model.
Results: Common bile duct stones were demonstrated in 43 out of 366 (12 per cent) patients. The most important predictors for demonstrable CBDS were: ultrasonography showing CBDS or bile duct dilatation, age > 60 years, fever, serum alkaline phosphatase level $> 670\,\mathrm{U\,L^{-1}}$, and serum amylase level $> 95\,\mathrm{U\,L^{-1}}$. In patients with a predicted probability of > 5 per cent, MRCP demonstrated CBDS in 10.6 per cent. In patients with a predicted probability of < 5 per cent, CBDS were demonstrated in 0.7 per cent.
Conclusion: The combination of this new predictive model with MRCP will minimize unnecessary ERCP and its subsequent morbidity in detecting CBDS in patients with gallstone disease planned for cholecystectomy.

Poster 29

Calcitonin precursors (CTpr): early markers of gut barrier dysfunction in patients with acute pancreatitis

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Aims: The aim of this study was to evaluate the role of plasma CTpr as an early marker of gut barrier dysfunction in patients with acute pancreatitis.

Methods: Intestinal permeability to macromolecules (polyethylene glycol 3350), serum endotoxin and antiendotoxin core antibodies, plasma CTpr and serum C-reactive protein (CRP) were measured on admission in 60 patients with acute pancreatitis. Attacks were classified as mild ($n = 48$) or severe ($n = 12$) according to the Atlanta criteria.

Results: Compared to mild attacks of acute pancreatitis, severe attacks were significantly associated with an increase in intestinal permeability index (median: 0.02 *versus* 0.006, $P < 0.001$), frequency of endotoxaemia (73 per cent *versus* 41 per cent, $P = 0.04$), and extent of depletion of serum IgM antiendotoxin antibodies (median 43 MMU *versus* 100 MMU, $P = 0.004$). Plasma CTpr levels were significantly elevated in patients with severe attacks compared to mild attacks on the day of admission and on day 3 (median 64 fmol mL^{-1} *versus* 22 fmol mL^{-1}, $P = 0.03$; and 90 fmol mL^{-1} *versus* 29 fmol mL^{-1}, $P = 0.003$, respectively). A positive and significant correlation was observed between the admission serum endotoxin and plasma CTpr levels on admission ($r = 0.7$, $P < 0.0001$) and on day 3 ($r = 0.96$, $P < 0.0001$), and between plasma CTpr on day 7 and the intestinal permeability index ($r = 0.85$, $P = 0.0001$). A weak positive correlation was observed between peak serum levels of CRP and plasma CTpr on admission ($r = 0.3$, $P = 0.017$) and on day 7 ($r = 0.471$, $P = 0.049$).

Conclusions: In patients with acute pancreatitis, plasma concentrations of CTpr appear to reflect the derangement in gut barrier function accurately rather than the extent of systemic inflammation.

Poster 30

An improved technique of pancreaticogastrostomy following pancreaticoduodenectomy

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Aims: Following partial pancreaticoduodenectomy, the pancreatic remnant can be implanted into the posterior wall of the stomach, however, this anastomosis is technically difficult to perform in the manner usually described. We present a method that facilitates construction of the pancreaticogastric anastomosis and assess its effect on the outcome.

Methods: All pylorus-preserving pancreaticoduodenectomies (PPPD) with pancreaticogastric reconstruction performed by a single surgeon from September 1991 to August 2000 were retrospectively analysed. From 1991 to 1997, the reconstruction was fashioned from behind, as is usually described, with a posterior single-layer anastomosis. From 1997 to 2000, the technique was modified by the addition of an anterior gastrotomy, and the pancreatic anastomosis was constructed via the gastric lumen. The outcome of patients in the two groups was compared.

Results: There were 51 PPPDs performed during the period of this study, the pancreaticogastrostomy was performed from outside the stomach in the first 31 cases, and via an anterior gastrotomy in the last 20 cases. There were three major anastomotic disruptions in the first group, while the second group experienced no major pancreatic leaks. There were no cases of bleeding from the suture line and no complications attributable to the anterior gastrotomy.

Conclusion: Following PPPD, the pancreaticogastric anastomosis can be conveniently fashioned via an anterior gastrotomy. This modification improves the field of vision, makes it easier to precisely suture the anastomosis and may contribute to a decrease in the rate of complications from this anastomosis.

Poster 31

Western body mass indices (BMI) need not compromise outcomes after modified D2 gastrectomy for carcinoma

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Aims: The western characteristics of advanced disease at presentation and an over-representation of elderly, unfit and overweight patients are often cited as the reasons for poorer outcomes than those described in Japan after radical D2 gastrectomy. The aim of this study was to determine the role of BMI in a western population on outcomes after modified D2 gastrectomy (preserving pancreas and spleen where possible) for gastric cancer.

Methods: Seventy-five consecutive patients undergoing an R0-modified D2 gastrectomy were studied prospectively. Thirty-four patients (median age 69 years, 22 M, median BMI: 26.0) classified as overweight with a high BMI by Japanese criteria (male BMI > 24.7, female > 22.6) were compared with 41 control patients (71 years; 27 M; BMI: 21.7) with a BMI below the above reference values.

Results:

	High BMI	Low BMI
ASA grade III (%)	12 (35)	20 (49)
Stages of disease I:II:III:IV	4:12:15:3	7:9:24:1
Operative time (min)	280 (182–390)	230 (145–355)**
Lymph node count	15 (12–31)	19 (12–32)*
Operative mortality (%)	3 (9)	3 (7)
Five-year survival (%)	57	54

*Median (range), **Pearson coefficient = 0.371, $P < 0.01$

Conclusion: Although 45 per cent of patients who were overweight required significantly longer operative times, outcomes were similar irrespective of BMI. Anaesthesia and surgery are clearly more hazardous and tedious in overweight gastric cancer patients, but this need not compromise the lymphadenectomy or influence the outcomes.

Poster 32

Early postoperative intraperitoneal chemotherapy (EPIC) for gastric carcinoma – a prospective study

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Aims: Survival for patients with serosa-positive gastric carcinoma remains poor predominantly as a result of peritoneal recurrence. The aim of this study was to determine the short- and long-term outcome following early postoperative intraperitoneal chemotherapy (EPIC).

Methods: From 1995 to 2001, 22 patients (median age 58 years, M:F = 1.4:1) received EPIC with aqueous mitomycin C (day 1) and 5-fluorouracil (days 2–4) after subtotal or total gastrectomy for serosa-positive gastric carcinoma. Early outcome and long-term survival was compared to 140 patients (median age 70 years, M:F = 2.9:1) who underwent surgery alone for transmural gastric carcinoma.

Results: EPIC was not completed for two patients (9 per cent) due to abdominal pain ($n = 1$) and leakage around the abdominal drain ($n = 1$). In-hospital mortality was comparable at 9.1 per cent (2/22) for EPIC and 7.8 per cent (11/140) for surgery alone ($P =$ NS). A further 23 per cent (5/22) of EPIC patients developed major complications, including one case of mitomycin C glomerulonephritis. Five EPIC patients had residual peritoneal disease and all died of persistent carcinoma within 12 months of surgery. Twenty-seven per cent (4/15) of EPIC patients without macroscopic residual peritoneal disease developed symptomatic loco-regional recurrence compared to 50 per cent (64/129) following surgery alone ($P = 0.09$). The 5-year survival of these patients was 44 per cent compared to only 18 per cent following surgery alone ($n = 129$) ($P = 0.14$).

Conclusions: EPIC is not associated with increased mortality after radical gastrectomy. A subgroup of patients experience excellent long-term outcome, and further investigation of this treatment modality is warranted within the confines of a randomized controlled trial.

Poster 33

Oesophageal stripping for postcorrosive stricture

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Aims: The aim of this study is to assess oesophageal stripping as a technique for benign strictures namely postcorrosive.

Methods: Between July 1997 and November 2001, 16 patients were operated upon for postcorrosive oesophageal stricture. All patients had long strictures ranging from 14 cm to the whole length of oesophagus and they all had failed medical and endoscopic treatment. A two-stage operation was performed including laparotomy, formation of gastric tube, pyloroplasty and feeding jejunostomy. Gastropharyngostomy was performed through a cervicotomy after stripping of the entire oesophagus using disposable vein stripper. Roux-en-Y pyloro-jejunostomy was performed in three patients because of pyloric stricture.

Results: All patients made complete recovery. Nine patients stayed one night in ITU, four patients spent two nights and three patients spent three nights. Three patients had anastomotic leak at the neck. Two of them presented with fistulae and the third one with a collection in the neck. These patients were treated conservatively nil by mouth, i.v. antibiotics, i.v. fluids and enteral feeding through jejunostomy. Follow-up of all 16 patients ranged from 2 months to 3 years, and none of them had residual dysphagia or chest pain.

Conclusions: Oesophageal stripping is to be recommended for postcorrosive strictures because of its simplicity, relative ease, safety and fewer postoperative complications.

Poster 34

Lack of availability of urgent endoscopic retrograde cholangiopancreatography (ERCP) may contribute to high early mortality from acute pancreatitis in Scotland

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Aims: The aim of the present study was to obtain a nationwide overview of death from acute pancreatitis (AP) in order to identify aspects of management amenable to modification.

Methods: A total of 63 deaths from AP were identified in Scotland by the Scottish Audit of Surgical Mortality (SASM) during the calendar year 2000. Cases were reviewed, and only deaths from AP were included in the analysis.

Results: There were 29 male and 34 female patients. The mean age was 73 years. The diagnosis of pancreatitis was made before death in 60 patients (95 per cent), in retrospect in one patient and at autopsy in two. Eight patients (13 per cent) had stratification of severity of AP documented in the case notes within 48 h of admission. Three patients could not be assessed, as they died immediately after admission. Out of 60 patients, 38 patients (63 per cent) underwent ultrasound scan of abdomen as part of their investigations. Twenty-nine patients (46 per cent) had a CT scan during their admission. Of 15 patients with a bilirubin of more than 40 μmol L^{-1}, four underwent ERCP that was completed successfully in two. The mean duration of in-hospital stay before death was 10.4 (1–64) days. Thirty-seven patients (59 per cent) died within the first 7 days of admission (early deaths), of these 30 (48 per cent) succumbed within 48 h, all as a result of multiple-organ dysfunction syndrome.

Conclusion: This study confirms the high proportion of early deaths from acute pancreatitis in Scotland and identifies the lack of availability of urgent ERCP as a potential contributory factor.

Poster 35

Persisting symptoms after treatment for achalasia: are clinicians diagnosing the problem?

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Aims: Achalasia can cause persisting symptoms after definitive therapy and often there is some uncertainty as to the diagnosis. This study retrospectively examined the data of 318 patients referred to the Oesophageal Unit and examined the mean time interval (MTI) between the initial diagnosis of achalasia to referral to the unit.

Methods: Over a 15-year period, 94 patients had therapy for achalasia and had persistent symptoms. Five patients were excluded for incorrect initial diagnosis. All patients underwent manometry, while 18 patients with reflux symptoms underwent ambulatory 24-h pH monitoring. Patients were analysed in three subgroups dependent upon treatment: balloon dilatation (group 1: $n = 56$; 30 males; mean age: 46 years), myotomy (group 2: $n = 7$; five males; mean age: 53 years) or both (group 3: $n = 26$; 11 males; mean age: 45 years). Prereferral diagnosis was compared to the diagnosis after investigation.

Results: The table below summarizes the study results. In all three groups, clinical referral diagnosis of under-treated achalasia was accurate (71/71). Post-treatment reflux was poorly diagnosed (8/18) and uncommon (8/89). Five patients with under-treated achalasia had coexistent post-treatment reflux. Total Ten of 18 patients with a prereferral diagnosis of post-treatment reflux had under-treated achalasia and no reflux.

Conclusion: Failure to thrive after treatment for achalasia is a difficult problem and the average referral time is long (all groups >56 months). Post-treatment reflux is uncommon (8/89) and tends to be overdiagnosed, whereas clinicians accurately diagnose under-treated achalasia (71/71).

		Pre-referral diagnosis accuracy (%)	
Patient groups	MTI (months)	Persistent achalasia	Post-treatment reflux
Group 1	56	100	40
Group 2	72	100	0

Poster 36

Laparoscopic *in situ* ablation (ISA) of liver cancer with cryotherapy and/or radiofrequency

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Aims: Curative resection is only feasible in 15–20 per cent of the liver cancers, and thus ISA may expand the indications for treatment. We describe cryotherapy (CSA) and radiofrequency ablation (RFA) for the treatment of irresectable liver cancers using a laparoscopic approach.

Methods: From January 1995 to December 2001, 38 patients with 146 lesions underwent ISA. Mean age was 62 years. Staging was by MRI and intraoperative ultrasound (IOUS). ISA was by CSA ($n = 9$), combination CSA + RFA ($n = 8$), RFA ($n = 15$), combination RFA + resection ($n = 6$). Adjuvant chemotherapy (5 FU/irinotecan) was given to 15/25 CRC patients. Pathology was colorectal cancer (CRC) metastases ($n = 25$), HCC ($n = 5$), neuro-endocrine ($n = 5$), melanoma ($n = 2$), renal cell carcinoma ($n = 1$). Complications of ISA and survival outcome calculated using Kaplan–Meier survival curves with log-rank test, with CRC patients compared with age-matched controls treated with systemic chemotherapy.

Results: Average follow-up was 26.6 months (range: 3–62 months; median: 26 months). Twenty-two patients are alive, 16 are deceased. Survival is significantly increased in ISA group compared to controls ($P < 0.0001$), and is consistent with both CSA ($P < 0.002$) and RFA ($P < 0.02$). Ablation site recurrence is 28 and 20 per cent, and new liver deposits 38 and 20 per cent with CSA and RFA, respectively. Procedure-related complications are higher with CSA (23 per cent *versus* 2 per cent). IOUS identified 10 per cent additional deposits *versus* MRI.

Conclusions: ISA is feasible with a laparoscopic approach and should include IOUS for accurate staging. ISA of liver cancer appears to have a survival benefit, and extends the therapeutic option in irresectable disease. A prospective randomized study of ISA in CRC metastases would seem reasonable.

Poster 37

The expression of TIMP-1 is a prognostic marker of poor outcome in patients with colorectal cancer liver metastases

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Aims: Matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) are enzymes involved in extracellular matrix breakdown and remodelling and have been implicated in tumour invasion and metastatic spread We investigated patients undergoing liver resection for colorectal cancer liver metastases to identify which enzymes could be significant prognostic markers

Methods: The expression pattern of MMPs (collagenase, MMP-1, gelatinase B: MMP-9, matrilysin MMP-7) and TIMPs (TIMP-1 and -2) was examined in formalin-fixed wax-embedded archival tissue using immunohistochemistry. Samples from 105 patients undergoing hepatic resection for colorectal cancer metastases during the period 1988–1996 were analysed.

Results: The expression pattern in the tumour immediately adjacent to the tumour/liver interface was examined The median survival was 3.2 years (95 per cent CI 2.3–4 1 years) for those patients who do not express TIMP-1 in tumour and 1 54 years (95 per cent CI 1 16–1 92 years) for those who did (log-rank test $\chi^2 = 3.54$, d f = 1, $P = 0.060$) When coexpression at the tumour/liver interface of TIMP-1 in the tumour and MMP-1 in the liver was analysed, the log-rank test indicated a significantly reduced survival for patients coexpressing these enzymes ($\chi^2 = 10\ 80$, d f = 4, $P = 0.029$). Neither the expression of MMP-1 alone nor any of the other enzymes predicted survival

Conclusion: TIMP-1 expression in colorectal cancer liver metastases, particularly in combination with MMP-1, is associated with poor outcome. TIMP-1 may be a useful prognostic marker

Poster 38

Risk-adjusted outcomes after oesophageal and gastric operations for cancer

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Aims: Centralization of surgery is at the core of proposed changes for service delivery in patients with oesophageal and gastric cancer Surgical case series are open to the criticism that they fail to define the characteristics of the surgical population within the context of the disease as a whole

Methods: The aim of this study was to prospectively measure short-term surgical outcomes in patients undergoing treatment for oesophageal and gastric cancers, using operation rates and a standardized scoring system (P-POSSUM) to define the surgical population.

Results: A total of 413 consecutive patients (223 oesophageal, 188 gastric) presenting to a single centre were evaluated For oesophageal cancer, 148 had operations with 140 (95 per cent) having resections aimed at cure Eighty-four (group A) underwent surgery alone and 56 (group B) had chemotherapy ± radiotherapy and surgery Group C (eight patients) had no resection. For gastric cancer, 74 had operations with 53 (72 per cent) having resections aimed at cure (group D) and 21 having palliative operations (group E). Actual and predicted morbidities and hospital mortalities were as follows.

	Morbidity (%)		Mortality (%)	
Group	Actual	Predicted	Actual	Predicted
A	69	73	6	9
B	73	69	12	7
C	25	39	0	3
D	51	59	4	6
E	43	52	14	7

Conclusion: There is a much higher resection rate for oesophageal cancer compared to gastric cancer P-POSSUM is an accurate predictor of morbidity in each group, but underestimates hospital mortality in patients undergoing multimodal therapy for oesophageal cancer and palliative surgery for gastric cancer

Poster 39

Does hospital size influence the outcome of patients with gastric cancer undergoing surgery?

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Aims: To determine whether hospital size to which patients with gastric cancer presented influenced operation rates Furthermore to establish whether there was a difference in operative mortality and survival between hospitals of different sizes performing gastric resections

Methods: Data from the Scottish Audit of Gastric and Oesophageal Cancer provided population-based data on the hospitals to which patients presented and underwent subsequent surgery. Follow-up data was complete for a minimum of 1 year Operation rates were compared for patients presenting to hospitals seeing <10 (band 1), 11–34 (band 2), 35–74 (band 3), and >75 (band 4) patients per year Hospital mortality and 1-year survival were compared according to the hospital throughput where surgery was performed

Results: The operation rates according to the hospital of presentation were as follows: 48 per cent· band 1, 51 per cent· band 2, 53 per cent. band 3, and 47 per cent band 4 The operative mortality and 1-year survival after surgery for high- and low-throughput hospitals are shown in the table

No resections per year	1–14	15–29	30–44	>45
Mortality and 1-year survival (%)	15 4	13 3	14 3	13 3

Conclusions: Although patients with gastric cancer in Scotland presented to 53 different hospitals of differing sizes, there was no evidence to suggest that the chance of operation was compromised by presentation to a smaller unit. Similarly, the in-hospital mortality and subsequent survival for patients with gastric cancer did not differ between hospitals of varying sizes performing the surgery

Poster 40

Telomerase expression and plasminogen activation in the progression of Barrett's oesophagus to adenocarcinoma

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Aims: Reactivation of telomerase and up-regulation of urokinase plasminogen activator (uPA) are important steps in the progression from Barrett's oesophagus to adenocarcinoma, resulting in the development of an immortal invasive phenotype The effect of telomerase expression on plasminogen activation during the development of Barrett's adenocarcinoma was examined

Methods: Samples of normal oesophageal mucosa (NOM, $n = 23$), Barrett's mucosa without dysplasia (NDB, $n = 14$), low-grade dysplasia (LGD, $n = 10$), high-grade dysplasia (HGD, $n = 10$) and oesophageal adenocarcinoma (OAC, $n = 30$) were obtained by biopsy Expression of telomerase and levels of uPA and its receptor (uPAr) were determined by ELISA The uPA and uPAr profiles of samples expressing telomerase were compared with those of telomerase-negative samples

Results: The uPA levels in normal mucosa and nondysplastic Barrett's were the same in telomerase-positive and telomerase-negative samples (NOM: 0.21 ng mg^{-1} *versus* 0.18 ng mg^{-1}, NDB 0.40 ng mg^{-1} *versus* 0 42 ng mg^{-1})

Expression of telomerase resulted in an increase in uPA expression early in the progression from low-grade dysplasia to carcinoma (LGD: 0.51 ng mg^{-1} *versus* 0.22 ng mg^{-1}; HGD: 0.51 ng mg^{-1} *versus* 0.6 ng mg^{-1}; OAC: 0.94 ng mg^{-1} *versus* 1.7 ng mg^{-1}). The uPAr levels were lower in telomerase-positive normal mucosa than telomerase negative (NOM: 0.7 ng mg^{-1} *versus* 1.2 ng mg^{-1}). Telomerase expression was associated with an increased expression of uPAr early in the progression from Barrett's to carcinoma (NDB: 5.2 ng mg^{-1} *versus* 2.0 ng mg^{-1}; LGD: 2.5 ng mg^{-1} *versus* 0.9 ng mg^{-1}; HGD: 1.3 ng mg^{-1} *versus* 1.6 ng mg^{-1}; OAC: 3.5 ng mg^{-1} *versus* 5.8 ng mg^{-1}).

Conclusions: This study demonstrates that telomerase expression is accompanied by increased expression of the components of the plasminogen activator system which are involved with invasion. Telomerase expression may therefore represent a novel marker for a more invasive phenotype in the development of oesophageal adenocarcinoma.

Poster 41

The role of laparoscopy with endoscopic ultrasound in preoperative assessment of patients with hepatobiliary malignancy

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Aims: Laparoscopic ultrasound is a relatively recent modality available for the surgeon in assessing resectability of a variety of hepatobiliary malignancies. In our unit, it has been routinely used for assessing colorectal metastases, cholangiocarcinomas and pancreatic carcinomas since 1998. The aim of this study was to demonstrate its accuracy in predicting operability, while its potential in avoiding unnecessary laparotomies over and above the more established radiological modalities of computerized tomography, angiography and ultrasound.

Methods: Between 1998 and 2001, 104 patients underwent laparoscopy with surgeon-operated endoscopic ultrasound. In 85 patients, the procedure was undertaken with intent to resect. In seven patients (6.7 per cent), laparoscopy was abandoned due to adhesions and the patient underwent laparotomy by default at a later date. For pancreatic cancer, liver and peritoneal metastases as well as invasion of the portal vein was assessed, whereas for colorectal liver metastases and cholangiocarcinomas, primarily liver and peritoneal involvement was looked for. The patient was deemed inoperable if any of these parameters were positive.

Results: Twenty-eight patients (36 per cent) were found to be unresectable at laparoscopy, avoiding unnecessary laparotomies. Fourteen patients (18 per cent) which were resectable at laparoscopy proved to be unresectable at later laparotomy. Of the 14 unnecessary laparotomies, on two patients palliative resection was contemplated and failed. Laparoscopy failed to diagnose portal vein involvement in nine patients (9/46 pancreas cancers = 20 per cent). In three patients, it failed to demonstrate significant liver metastases (3/78 = 3.8 per cent). In four patients, laparoscopy missed peritoneal metastases (4/78 = 5 per cent).

Conclusions: Overall, laparoscopy correctly staged 82 per cent of patients in which the procedure was deemed successful.

Poster 42

Public perception of the significance of dysphagia

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Aims: Overall survival for oesophageal carcinoma is at best 10 per cent. Two-thirds of patients have symptoms for more than 3 months suggesting a lack of awareness of the sinister significance of dysphagia.

Methods: A survey was conducted in the community using a questionnaire designed to evaluate the subjects' impression of the significance of dysphagia and compare it with their perception of the significance of a breast lump.

Results: There were 340 subjects interviewed. Seventy-five per cent stated they would attend a doctor within 1 week of developing dysphagia compared with 86 per cent who would seek attention for a breast lump ($P < 0.05$, χ^2, 1 d.f.). Only 14 per cent felt that a cancer was a probable cause for dysphagia *versus* 79 per cent who felt the same for a breast lump ($P < 0.0001$, χ^2, 1 d.f.). Only 43 per cent felt that specialist referral was warranted for dysphagia, whereas specialist referral was expected by 92 per cent for breast lumps ($P < 0.0001$, χ^2, 1 d.f.). The commonest cause of dysphagia was considered to be an infection (63 per cent). Ninety-six per cent had heard breast cancer discussed in the media compared to only 28 per cent for oesophageal carcinoma ($P < 0.0001$, χ^2, 1 d.f.).

Conclusions: There is evident need of an oesophageal cancer-awareness programme if patients are to present at an earlier stage of their disease. Whether this will translate to improved prognosis remains to be determined.

Poster 43

Endoscopic ultrasound as a primary modality for diagnosis of common bile duct stones – a prospective study

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Aims: ERCP is the most common modality used for the diagnosis and treatment of common bile duct (CBD) stones, but the pickup rate is low and there is a risk of acute pancreatitis. The aim of this study was to prospectively assess the efficacy of endoscopic ultrasound (EUS) as the primary diagnostic modality for CBD stones.

Methods: Forty-four patients were entered into the study. All patients had presented with biliary colic or acute pancreatitis and were found to have deranged liver function or a dilated CBD on abdominal ultrasound. All patients underwent EUS as the primary diagnostic modality. Those with a finding of CBD stones on EUS underwent an ERCP and stone extraction, followed by a laparoscopic cholecystectomy (LC). Those with a negative EUS had an intraoperative cholangiogram (OTC) during their LC. All EUS procedures were performed by a single gastroenterologist.

Results: CBD stones were found in 14 patients (31.8 per cent) at EUS. At ERCP, nine of these were found to be true positives (64.2 per cent) and five were false positives (35.8 per cent). Of the remaining 30 patients who underwent LC with OTC, two patients (6.6 per cent) were found to have CBD stones which were dealt with by postoperative ERCP. Twenty-eight out of 44 patients (63.6 per cent) were found to be true negatives and were thus spared an unnecessary ERCP.

Conclusion: EUS is effective as a primary modality for diagnosis of CBD stones, but it is operator dependent and has a long learning curve.

Poster 44

Characterization of familial pancreatic cancer kindreds on the European registry of hereditary pancreatitis and familial pancreatic cancer (EUROPAC)

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Aims: In 10 per cent of the cases of pancreatic cancer, there is a familial background to the disease. Mutated genes predisposing to pancreatic cancer syndromes include *PRSS1* (associated with hereditary pancreatitis), *STK11*, *MLH1*, *BRCA2* and *p16INK4a*. However, the gene responsible for the great majority of familial pancreatic cancers (FPC) is not known. Discovering the gene(s) responsible for FPC will enable presymptomatic diagnosis and enhance our understanding of the molecular pathology of pancreatic cancer. EUROPAC has one of the largest collections of FPC families. Our immediate objective is to characterize the families so that we can carry out segregation analysis.

Methods: After ethical committee approval, EUROPAC recruited 113 families with pancreatic cancer (223 affected individuals), of which 84 families were characterized purely by pancreatic cancer. Affected individuals were tested for *BRCA2* and *STK11* mutations. Clinical details are stored on an anonymized database. Statistical analysis was carried out with StatView version 5.

Results: For the pure pancreatic cancer families, median age of onset of cancer was 59 (IQR: 51–68), $N = 88$. There was no significant gender difference in the age of onset or transmission (6 male–male, 6 male–female, 11 female–male, 13 female–female). In 4 of the 84 families, there was an individual with apparent nonpenetrance to age 70 years (assuming an autosomal dominant disease).

There was anticipation of age of onset with affected children developing cancer a median of 15 years earlier than their parents (IQR 4.5–21).

Conclusion: Our results are consistent with an autosomal dominant mode of inheritance with some evidence of anticipation.

Poster 45

Potentially problematic common bile duct calculi in patients selected for laparoscopic cholecystectomy

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Aim: To evaluate the early natural history and true incidence of problematic common bile duct calculi in patients undergoing laparoscopic cholecystectomy (LC).

Methods: We evaluated the true incidence of potentially problematic common bile duct calculi in patients undergoing LC by routine operative cholangiography, and where positive by repeated tube cholangiography at 48 h and 6 weeks postsurgery. Where positive at 6 weeks patients underwent stone extraction by ERCP.

Results: Operative cholangiography was accomplished in 986 (95 per cent) of 1041 consecutive patients. Fifty-one (5.1 per cent) had at least one radiographic filling defect in the common bile duct and the fine-bore cholangiogram catheter was left in situ (there was no correlation with liver function tests or clinical evaluation). Sixteen of these (31 per cent) had a normal cholangiogram at 48 h (false-positive operative cholangiograms) and a further 13 (25 per cent) had a normal cholangiogram at 6 weeks (spontaneous passage of calculi). Only 22 patients (2.2 per cent) required ERCP for persistent calculi at 6 weeks post-laparoscopic cholecystectomy.

Conclusions: Common bile duct calculi passed spontaneously in more than one-third of the affected patients within 6 weeks of LC, leaving a potentially problematic stone rate of <2.5 per cent. These data support a policy of expectant management of choledocholithiasis and that routine operative cholangiography is unnecessary.

Poster 46

New technique for liver resection using heat coagulative ablation

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Aims: Intraoperative blood loss during liver resection remains a major concern because of its association with higher postoperative complications and shorter long-term survival. The aim of this study is to assess the feasibility and safety of a novel concept for liver resection using a radiofrequency (RF) energy-assisted technique for anatomical- and nonanatomical-orientated segmental liver resection of hepatic tumours.

Methods: From January 2001 to December 2001, 24 patients with various hepatic tumours were operated on using RF energy-assisted liver resection. RF energy was applied along the resection edge to create 'zones of desiccation' prior to resection with a scalpel.

Results: Median resection time was 45 min (range: 30–110). The median blood loss during resection was 30 mL (range: 15–992) and mean preoperative and postoperative haemoglobin values were 13.7 g dL^{-1} (SD + 1.68) and 11.79 g dL^{-1} (SD + 1.43), respectively. No blood transfusions were required, nor was any mortality observed. There were two postoperative complications, one subphrenic abscess and one chest infection. Median postoperative stay for patients without complications was 8 days (range 5–25). No liver recurrence was detected in patients undergoing resection with this technique during follow-up (median 7, range 1–12 months).

Conclusions: Anatomical and nonanatomical segmental liver resection assisted by RF energy is feasible, easy and safe. This novel technique offers a new method for 'transfusion-free' resection without the need for sutures, ties, staples or tissue glue.

General

Poster 47

Randomized prospective trial of bilayer prolene hernia system (PHS) *versus* lichtenstein mesh for inguinal hernia repair under local anaesthesia

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Aims: To compare the short-term results of the bilayer prolene hernia system mesh (Ethicon, Inc) with the onlay lichtenstein mesh.

Methods: Sixty-four patients were randomized to undergo either a PHS or a lichtenstein inguinal hernia repair under local anaesthetic as a day case procedure. Outcome measures were duration of surgery, volume of local anaesthetic used, time to discharge, pain scores on day one, and overall, analgesic requirements, complications, time to return to work, driving and full activity, and satisfaction scores.

Results: No patient required sedation or conversion to general anaesthesia. Mean duration of surgery in the PHS group was 36 min (SD = 11) *versus* 34 min lichtenstein (SD = 8, $P = 0.3$). Mean volume of local anaesthetic was 64 mL (SD = 18) for PHS and 62 mL (SD = 17) for lichtenstein ($P = 0.8$). There was no significant difference in analgesic requirements (25 tablets *versus* 11 tablets total, $P = 0.65$). Twelve per cent of patients did not require analgesia. Overall mean pain score was 3.5/10 *versus* 2.5/10 (visual analogue scale, $P = 0.1$). Mean time to return to work was 42 days *versus* 30 days ($P = 0.3$), returning to driving was 20 days *versus* 14 days ($P = 0.2$) and full activity 21 days *versus* 22 days ($P = 0.8$). Patient satisfaction was very high with both techniques.

Conclusions: This trial confirms that the PHS mesh technique is a safe procedure that takes no longer to perform than the standard lichtenstein approach. The preperitoneal dissection in particular is well tolerated under local anaesthesia. Long-term follow-up of these patients will be performed to establish the recurrence rate.

Poster 48

The effect of port composition on tumour cell adherence: an *in vivo* model

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Aims: *In vitro* models demonstrate that increased number of tumour cells are deposited both on metal ports when compared with plastic ports and on the port sites through which these have passed. Therefore, the *in vivo* deposition of such cells to ports and port sites was investigated.

Methods: LIM 1215 tumour cells were injected under direct vision into the pelvises of 16 female pigs of 30 kg (range (15–70) × 10^6 cells). A total of six metal and six plastic ports were inserted through each anterior abdominal wall and were either left in situ for 30 min (nondisplaced) or were twice removed and replaced through the original wound (displaced).

Results: Increasing the tumour cell inoculum resulted in increased deposition of tumour cells on both ports ($P = 0.002$) and on port sites ($P = 0.017$). Significantly more tumour cells adhered on metal ports when compared with plastic ports ($P = 0.04$), although this failed to reach significance for the metal port sites ($P = 0.066$). Although displacement of ports did not increase the number of tumour cells adherent to ports ($P = 0.45$), this did result in more tumour cells being deposited on the port sites ($P = 0.01$).

Conclusions: These data suggest that minimizing the number of tumour cells within the abdominal cavity, employing plastic ports and securing ports to prevent inadvertent displacement would be expected to reduce the number of tumour cells deposited in port sites during operative laparoscopy. This may be beneficial in reducing the incidence in port site metastases after laparoscopic surgery for gastrointestinal malignancies.

Poster 49

Oral administration of carbohydrate-containing fluids prior to major elective surgery preserves lean tissue postoperatively

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Aims: To assess the tolerance of preoperative carbohydrate fluid administration and the postoperative metabolic and clinical response.

Methods: A double-blind randomized study recruited major surgical patients to receive either placebo drink or carbohydrate (12.6 g per 100 mL) drink (CHOD). Patients consumed 800 mL the evening before and 400 mL 2–3 h before anaesthesia. Nutritional status was measured preoperatively, postoperatively and at discharge. Body mass index (BMI), arm anthropometry, blood glucose and insulin concentrations were measured pre- and postoperatively. Length of stay (LOS) and complications were recorded.

Results: Thirty-four controls and 31 CHODs completed the study. Groups were comparable in gender, age (control: 52.1 + 2.4 years *versus* CHOD: 52.8 + 2.5 years), body mass (control: 25.1 + 1.7; CHO: 25.2 + 1.2) and procedure. Loss of muscle mass (arm muscle circumference) was significantly greater in the control group (control: 3.9 + 0.6 per cent *versus* CHOD: 1.9 + 0.6 per cent; $P < 0.05$). Baseline insulin (control: 20.7 + 4.9 mU L^{-1}; CHOD: 24.6 + 6.2 mU L^{-1}) and glucose (control: 6.0 + 1.4; CHOD: 5.7 + 1.4 mmol L^{-1}) were comparable and did not differ postoperatively. No complications were recorded as a result of fluid consumption. Clinical complications were recorded in six patients from each group. LOS in controls was 10 days (IQR = 6) and 8 days in CHOD (IQR = 4).

Conclusions: A CHO energy source attenuates depletion of muscle mass after surgery, but mechanisms involved remain unclear. These results give credence to the proposal for evaluating current practice of fasting prior to surgery and confirm aspiration risk to be minimal.

Poster 50

Comparison of bench test evaluations of surgical skill with live operating performance assessments

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Aim: Attempts at assessing surgical proficiency have generally used laboratory simulation to evaluate skill. The aim of this study was to observe how closely technical ability as measured on bench simulation compared to actual operative performance.

Methods: Twenty-two general surgeons and trainees were recruited: consultant ($n = 4$), specialist registrar ($n = 14$) and SHO ($n = 4$). They were assessed whilst performing a saphenofemoral dissection on an anaesthetized patient in the operating theatre, as well as on an inanimate model of this same procedure within the laboratory. The objective structured assessment of technical skill (OSATS) method, consisting of a seven-parameter global rating (maximum score: 35), and 17 point step-by-step checklist (maximum score: 17) was used to measure performance in both environments. Face, content and construct validity of the synthetic model had already previously been established.

Results: There was a significant relationship between technical skill as measured on the bench test model with performance within the operating theatre with respect to both global rating (Pearson correlation coefficient: 0.824; $P < 0.001$; α coefficient: 0.89) and checklist rating ($r = 0.514$; $P < 0.02$; α coefficient: 0.68) assessments. Global rating scores correlated with experience for both operative ($r = 0.822$; $P < 0.001$) and bench ($r = 0.748$; $P < 0.001$) settings. There was no difference in the level of measured performance between operating theatre and bench model (global rating mean 23.25 ± 6.66 *versus* 23.75 ± 5.62, respectively, paired *t*-test $P = 0.559$).

Conclusions: Assessment of technical skill using inanimate procedural simulation translates to actual surgical performance within the operating theatre. This further validates the use of bench test evaluations to measure surgical technical ability.

Poster 51

A randomized controlled trial of acute normovolaemic haemodilution (ANH) in gastrointestinal surgery

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Aims: To assess the effects of ANH on allogeneic transfusion, postoperative complications and length of stay.

Methods: Patients scheduled for major gastrointestinal surgery were randomized to 3-unit ANH, or no ANH. Both groups underwent identical management including adherence to a transfusion protocol. Outcome measures included the number of patients receiving allogeneic blood, complications and length of stay.

Results: A total of 316 consecutive patients were screened, of which 160 (51 per cent) were eligible; median age: 62 years (range 23–90); 'ANH': $n = 78$; 'no ANH': $n = 82$. There was no significant difference between groups in the number of patients receiving allogeneic blood 23/78 (29 per cent) *versus* 25/82 (30 per cent), the total number of allogeneic units transfused (92 *versus* 93), complication rate or length of stay. Haemodilution significantly increased anaesthetic time; median: 55 min (range 15–90) *versus* 40 min (range 17–80) ($P < 0.001$). Significantly less patients in the ANH group experienced oliguria in the immediate postoperative period 37/78 (47 per cent) *versus* 55/82 (67 per cent) ($P = 0.012$). The most significant factors effecting transfusion are blood loss (e.g. >1000 mL OR 13.37 (3.22–55.54 *versus* <500 mL)), starting haemoglobin (OR 0.43 (0.28–0.65)), and age (OR 1.03 (1–1.07)). When compared with ASA-matched historical controls, the introduction of a transfusion protocol reduced the transfusion rate from 123/317 (39 per cent) to 48/160 (30 per cent) ($P = 0.058$).

Conclusions: In this large definitive study, ANH did not affect allogeneic transfusion rate in major gastrointestinal surgery. Preoperative haemoglobin, blood loss and transfusion protocol are the key factors influencing allogeneic transfusion.

Poster 52

The impact of laparoscopic appendicectomy on training

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Aims: Open appendicectomies were the mainstay training operations for junior surgeons. The impact of change to a laparoscopic approach on trainees and trainers has not been assessed. We aimed to assess the impact of laparoscopic appendicectomy as compared to open appendicectomy on various trainee grades and the consultant commitment required to bring about this change. The resultant complication rates were assessed.

Aim: We aim to assess the impact of laparoscopic appendicectomy as compared to open appendicectomy on various trainee grades and the consultant commitment required to bring about this change. The resultant complication rates were assessed.

Methods: A total of 176 laparoscopic appendicectomies were compared to 132 open appendicectomies. The level of the surgeon performing the operation, the amount of consultant input and complications were recorded. Senior house officers (SHOs), registrars inexperienced (RI) in laparoscopic surgery (performed laparoscopic surgery for less than 1 year) and registrars experienced (RE) in laparoscopic surgery were studied.

Results: Sixty-seven (51 per cent) of all open appendicectomies were performed assisted by SHOs as compared to 60 (46 per cent) performed by registrars; seven (5 per cent) were performed by a consultant and 12 (9 per cent) were in a supervisory role. Only nine (5 per cent) laparoscopic appendicectomies were performed assisted by SHOs as compared to 158 (90 per cent) of cases performed by registrars; nine (5 per cent) were performed by consultants and 12 (7 per cent) were in a supervisory role. The open approach had 13 (10 per cent) complications, of which eight were wound infections, four were pelvic abscesses and one was a chest infection. There were 16 (9 per cent) complications with laparoscopic appendicectomies, of which eight were wound infections, three were pelvic abscesses and five were urinary or chest infections.

Conclusions: Laparoscopic appendicectomy has a major impact on junior surgical trainees, but not on senior staff.

Poster 53

Surgical workload at community and private hospitals

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Aims: To determine the contribution of community and private hospitals to the elective workload of a district general hospital (DGH) surgeon.

Methods: All elective operations performed at the DGH, community and private hospitals were recorded for 3 years (1999–2001). Royal College of Surgeons of England Intermediate Equivalents were also calculated. The DGH was working at capacity. Cancellations due to lack of beds and operating times were common.

Results:

1. A mean of 190 operations (21 per cent) were performed at the DGH, 526 (57 per cent) at the community hospitals and 199 (22 per cent) at the private hospitals.
2. Out of the total of 526, 716 NHS operations (73 per cent) were performed at the community hospital.
3. A mean of 302 intermediate equivalents (29 per cent) were performed at the DGH, 490 (47 per cent) at the community hospitals and 239 (23 per cent) at the private hospitals.

Conclusions:

1. At the DGH, only 21 per cent of all elective operations were performed, albeit more major cases.
2. Without the community hospital, nearly three-quarters of the NHS patients would not have had their operations.
3. Without the private hospitals, an extra 200 patients year^{-1} would require NHS treatment.

Poster 54

Incidence of neuropathic pain in patients presenting with chronic pain after groin hernia repair

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Aim: To determine the incidence of neuropathic pain in patients presenting with chronic pain after groin hernia repair.

Methods: Patients had undergone a laparoscopic or open-mesh repair of groin hernia over a 2-year period. Those who complained of chronic pain and limitation of physical activity were invited to attend an outpatient clinic. Assessment was jointly carried out by a general surgeon (SK) and a pain medicine specialist (LC). Diagnosis of neuropathic pain was based on a history of episodes of burning, shooting pains in the groin and signs of altered sensation. Specifically, evidence of dynamic allodynia to cotton wool or mechanical hyperalgesia to pin prick was sought.

Results: A total of 112 patients complained of persistent pain (median duration: 33.5 months; range: 21–44) and limitation of physical activity were evaluated. Sixty-eight (60.7 per cent) patients, 62 males and six females (median age: 61.4 years; range: 26–86) were evaluated. Of those not seen, pain had resolved in 24/112 (21.4 per cent) patients and 20/112 (17.8 per cent) patients did not reply. A total of 23/68 (34.3 per cent) patients were diagnosed to have neuropathic pain, and 4/68 (5.9 per cent) had developed a recurrent or a contralateral hernia.

Conclusion: Clinical assessment found 23 patients with neuropathic pain. If it is assumed that the patients not attending did not have neuropathic pain, then the overall incidence of neuropathic pain in patients with persistent pain after groin hernia repair was 20.5 per cent (23/112).

Poster 55

The effect of a second-generation three-dimensional visualization system on laparoscopic dexterity

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Aims: Binocular visual perception is impaired in conventional laparoscopic surgery, since the surgeon views a two-dimensional (2D) image on a flat screen, and although logic suggests that effective restoration of 3D vision might increase operative dexterity, this has never been proven in the surgical setting itself. This trial utilizes a second-generation three-dimensional (3D) system (Autovision 150, Surgical Vision Ltd, Reading, UK) on a laboratory-based simulation of a laparoscopic cholecystectomy using fresh cadaveric pig livers employing motion analysis as a measure of dexterity.

Methods: Each of the seven surgeons performed 10 cases on this model, each operation being randomized to 2D or 3D, so that subjects utilized each system in five cases in random order. Dexterity throughout individual subsections of each procedure was assessed using motion trackers (Polhemus IsotrackII) on the surgeons' hands. Motion scores were compared for individual surgeons performing in 2D and 3D, using within-subjects analysis for each surgeon, controlling for repetition.

Results: Results are shown in Tables 1 and 2.

Table 1. Mean (±SEM) values

	2D/3D	Time (s)	Distance (cm)	Speed (cm s^{-1})	Movements (number)
Calot's dissection	2D	212.9 (17.3)*	481.1 (55.7)*	2.4 (0.2)	153.6 (13.9)*
	3D	141.5 (9.9)*	300.2 (32.6)*	2.1 (0.2)	101.6 (8.2)*
Placing three clips	2D	20.0 (0.6)*	32.2 (2.5)*	1.6 (0.1)	12.8 (0.6)*
	3D	15.5 (0.6)*	28.3 (3.4)*	1.7 (0.1)	9.8 (0.6)*
Cutting duct/artery	2D	11.3 (0.7)*	15.4 (1.3)	1.4 (0.1)	7.1 (0.6)
	3D	9.2 (0.5)*	13.6 (1.3)	1.4 (0.1)	5.8 (0.5)
GB dissection	2D	556.9 (33.5)*	1642.6 (156.0)*	2.9 (0.2)	434.1 (34.9)*
	3D	459.4 (25.8)*	1352.8 (120.6)*	2.8 (0.2)	351.7 (27.0)

*Significant differences: 2D *versus* 3D (Wilcoxon).

Table 2. Levels of significance

	Time (s)	Distance (cm)	Speed (cm s^{-1})	Movements (number)
Calot's dissection	0.000	0.001	N/S	0.000
Placing three clips	0.000	0.001	N/S	0.000
Cutting duct/artery	0.03	N/S	N/S	N/S
GB dissection	0.001	0.006	N/S	0.001

Conclusions: This study of 70 laparoscopic cholecystectomies on a validated simulation on a pig model has shown that when assessing dexterity objectively, major advantages in terms of laparoscopic dexterity can be seen when operating with a new second-generation 3D visualization system compared with standard 2D. If such systems are acceptable in terms of cost and user comfort, these increases in dexterity might transfer into improvements in operative ability.

Poster 56

Management and outcome of liver trauma

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Aims: Nonoperative management has been applied increasingly in the treatment of liver trauma. This study reviews experience with liver trauma at a specialist unit.

Methods: From January 1990 to December 2001, 35 patients (27 male) were admitted to a specialist centre with traumatic hepatic injuries. Mean age was 31 years. Thirty-two patients sustained blunt abdominal trauma and three patients sustained penetrating trauma.

Results: Twenty-three patients were managed conservatively. Twelve patients were managed operatively and three of those had liver resection. Eight patients underwent laparotomy at source hospital. Five of those eight underwent a further laparotomy in our unit, three for removal of packs, two for unexplained sepsis. Four patients admitted directly had laparotomy. Twenty-two patients made a satisfactory recovery. Four patients died: one died on the day of the trauma; two died at 15 days and the fourth died at 21 days. One patient had percutaneous drainage of a haematoma compressing his biliary tree. One patient developed an ischaemic stricture of his common bile duct, necessitating hepaticojejunostomy. One patient was transferred for neurosurgical care.

Conclusions: The majority of patients can be managed conservatively nowadays and avoid liver resection, which was traditionally the preferred management of these injuries. Surgical management is reserved in the early period for patients who remain haemodynamically unstable, and in the late period for suspected hepatic devascularization as a cause of sepsis.

Poster 57

Validation of a simple, automated test for catheter-related sepsis (CRS)

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Aims: Endoluminal brushing is an *in situ* technique, which has been shown to be highly accurate in the diagnosis of CRS, but it is expensive and can be technically demanding. The differential time to positivity (DTP) of central to peripheral blood has been suggested as a simple, automated and equally accurate method of diagnosing CRS. This study directly compares this technique with the gold standard of endoluminal brushing.

Methods: Central venous catheters (CVCs) were assessed on suspicion of causing CRS. Central blood cultures were drawn from each CVC, with a simultaneous set of peripheral cultures and analysed by automated qualitative method (Vital^{R}). Each CVC then underwent endoluminal lumen brushing. A positive endoluminal brush result was considered to be the growth of >100 cfu mL^{-1}, and a DTP of >2 h between time to positivity of central to peripheral cultures was considered a positive result.

Results: There were 115 cases of suspected CRS (median dwell time: 9 days (IQR: 6–14) for uncuffed CVCs; 105 days (IQR: 59–233) for Hickman lines). Thirty-five cases of CRS were identified on endoluminal brushing. The DTP method confirmed 23 of these cases. There were three false-positive results. The sensitivity of the DTP technique was therefore 65 per cent with a specificity of 96 per cent.

Conclusions: DTP offers a simple, automated test for CRS, which is highly specific and does not require CVC removal.

Poster 58

The prevention of peripheral vein thrombophlebitis (PVT) in surgical patients receiving peripheral intravenous nutrition via a fine-bore catheter

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Aims: Fine-bore catheters are all known to decrease the incidence of PVT when used for full peripheral intravenous nutrition, and catheter survival can be extended further with the addition of both heparin and hydrocortisone to the feed. The effect on fine-bore catheter survival of either heparin or hydrocortisone alone has not previously been assessed, and this study addresses the need for the addition of both drugs.

Methods: Randomized double-blind trial in surgical patients fed full peripheral intravenous nutrition (1200 mosm kg^{-1}). Each had a fine-bore 22 Ch catheter inserted via antecubital fossa vein. The upper limb was examined daily, and the catheter was removed on development of PVT or on completion of feeding. Patients were then randomized into three groups.

- Group 1 with heparin 1500 U and hydrocortisone 15 mg.
- Group 2 with heparin 1500 U alone.
- Group 3 with hydrocortisone 15 mg alone.

Total catheter survival was represented as Kaplan–Meier survival curves with statistical difference assessed using log-rank statistics.

Results: A total of 123 patients were recruited (M:F = 63:60; median age: 67 years (IQR: 53–77)). Total catheter survival displayed in the table below.

	Median survival in days (confidence intervals)	Difference between groups
Group 1	9 (6–11)	$P = 0.59$
Group 2	7 (4.7–9)	$P = 0.0015^*$
Group 3	5 (3.7–6)	$P = 0.03^*$

*Statistical significance.

Conclusions: The addition of heparin has a significant role in fine-bore catheter survival when added to feeds providing full intravenous nutrition. Combining it with hydrocortisone appears to offer little or no significant benefit.

Poster 59

Excellence in surgery: are changes in training compatible with this goal?

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Aims: To compare the surgical exposure of two senior trainees, with the same subspecialty interest, at the same hospital in 1993 and in 2000.

Methods: An audit of prospectively collected logbook data from 9 months in 1993 and 9 months in 2000.

Results: The trainee in 2000 did more large bowel resections (subspecialty interest) and 'other' laparotomies (small bowel resection, division of adhesions) than the trainee in 1993; however, the 1993 trainee did more procedures outside of their subspecialty interest when compared to the 2000 trainee (see Table).

	Large bowel resection	Other laparotomy	Upper GI resection	General[a]	Cholecystectomy (open)	Hernia
1993	36	22	13	74	27 (9)	56
2000	45	29	4	2	16 (2)	34

[a] Thyroid surgery, varicose vein surgery, breast surgery.

The trainee in 1993 had six theatre sessions and two outpatient sessions a week, the 2000 trainee had 3.5 theatre sessions and two outpatient sessions a week.

Conclusions: The opportunity for performing surgical procedures has decreased; however, the senior surgical trainee of today has a good exposure to cases of their subspecialty interest in their final years, but is relatively poorly exposed to procedures outside of their subspecialty interest. This may have implications to the future provision of services and covering of the general surgical on-call.

Poster 60

Short-term outcome of inguinal hernia repair: comparison between standard tension-free lichtenstein repair *versus* prolene hernia system (PHS) repair

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Aims: To compare in a randomized prospective study tension-free Lichtenstein mesh repair with prolene hernia system (PHS) repair.

Methods: One hundred patients were randomized to Lichtenstein repair and PHS repair. Patients with recurrent or irreducible hernia were excluded from the study. All procedures were performed by one surgeon, as a day case, under local anaesthesia Following surgery, patients were asked to score their pain on visual analogue pain scales and document the amount of pain killers taken in the postoperative period. All complications were recorded by the patients. Follow-up took place at 1 week, 1 month and 3 months after the operation

Results: Forty-five Lichtenstein repair and 55 PHS repairs were performed. The groups were comparable in terms of age and comorbidity Haematoma and wound infection rates were similar in both groups There was no difference in postoperative pain scores or analgesia consumption in the postoperative period. Median time of surgery was significantly shorter in PHS group (46 min *versus* 53 min, $P = 0.005$). Return-to-normal activities in the PHS group was quicker (6 days *versus* 8.9 days; $P < 0.00005$). Return to work was earlier in the PHS group (14 days *versus* 23 days; $P < 0.00005$).

Conclusions: PHS mesh repair, which is simple to perform under local anaesthesia on a day case basis, takes less time to perform and results in quicker return-to-normal activities and work.

Vascular

Poster 61

Audit of general practitioners' referral policy and risk factor management in patients with intermittent claudication

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Aims: Intermittent claudication (IC) is common and patients have a risk of developing or dying from coronary heart disease equivalent to that of patients surviving their first myocardial infarction The aim was to determine general practitioners' (GPs) attitudes to referral, risk factor management and treatment in patients presenting with life-style limiting IC.

Methods: Postal survey questionnaire of 334 GPs regarding smoking, exercise, antiplatelet therapy, diabetes and cholesterol. A similar survey was performed in 104 new clinic patients with IC.

Results: A total of 242 (73 per cent) GPs responded At initial patient presentation, 50 per cent would manage risk factors and reassess and 45 per cent would treat risk factors and refer directly to the clinic. Ninety per cent would prescribe aspirin, 99 per cent advise smoking cessation and 89 per cent advise increase in exercise Blood glucose would be checked by 77 per cent and cholesterol by 85 per cent. The cholesterol levels (mmol L^{-1}) that GPs would treat are: <5, 5 per cent; >5, 36 per cent; >5.5, 18 per cent; >6, 23 per cent and 18 per cent would consider the patients' overall risk factor profile Initial management was diet alone in 70 per cent, statin in 30 per cent and 18 per cent had an age limit. In clinic patients, 62 per cent were prescribed aspirin, 81 per cent had an elevated cholesterol and less than half were on treatment.

Conclusion: Cholesterol-lowering therapy is required in 80 per cent of claudicants, yet it is considered to be primary prevention by 18 per cent of GPs and the majority would only treat levels above 5.5 mmol L^{-1}. Recent data suggests that claudicants should be treated with a statin irrespective of their cholesterol level, and thus national guidelines to ensure adequate secondary prevention of claudicants are urgently required.

Poster 62

An audit of varicose vein surgery

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Aims: A large number of patients are operated upon for varicose veins, yet they are rarely routinely followed up. We therefore wanted to find out whether best practice was being followed and the patients were benefiting from their surgery.

Methods: The notes of 247 patients treated in 1998/1999 were audited, in addition a locally developed questionnaire incorporating the EQ5D quality-of-life questionnaire was administered to all patients.

Results: Only 40 per cent of the patients with primary varicose veins had hand-held Doppler examination, only 65 and 80 per cent of patients with short saphenous reflux and recurrent varicose veins, respectively, had duplex assessment. In 50 per cent of cases, the long saphenous vein was not stripped; 56 per cent of patients with recurrent varicose veins were operated on by nonvascular consultants or trainees. In only 50 and 30 per cent were the SHOs and SpRs, respectively, supervised. The case note complication rate was only 10 per cent, but the patient-reported complication rate was 65 per cent. Seventy-two per cent of the patients were satisfied with their treatment and 65 per cent had an improvement in QOL score. Surgery by a nonsupervised trainee and nonvascular consultants had a statistically significant effect on recurrence.

Conclusions: Compliance with standards of best practice fell short, however, the majority of patients were happy with their treatment. A local policy on the treatment of varicose veins is to be developed incorporating the above findings and will be reaudited in the near future.

Poster 63

The relationship between socioeconomic status, symptomatic carotid territory disease and carotid endarterectomy

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Aims: Recent evidence suggests a strong association between socioeconomic status and atherosclerosis, but little information exists on the relationship between socioeconomic status, symptomatic carotid disease (SCD) and rates of carotid endarterectomy (CEA). The aim of this study was to evaluate the Carstair Deprivation Score (CDS) of patients admitted with SCD and those undergoing CEA in one health board.

Methods: The CDS score was determined for all patients admitted with SCD (ISD 9 codes 433.1, 433.09, 435, 437.1; ICD-10 165.2, 163, 163.2, G45.1, G45.3, G45.9) and those undergoing CEA between 1 April 1995 and 31 March 2000. Expected and actual rates for each of the CDS (1–7) were determined by direct and indirect methods of standardization assuming equal distribution allowing for age and sex Results were analysed using the Mantel–Haenszel test Only first-time admissions and CEA were included.

Results: A total of 1203 patients were admitted with SCD. The admission rates of symptomatic patients were less than expected in the more affluent group (Carstair 1, $P < 0.005$) and significantly higher in the most deprived group (Carstair 7, $P < 0.001$). There were no differences between the expected and actual rates of CEA and CDS in 192 patients. CEA rates tended to be higher in the most affluent group, who had the lowest prevalence of disease.

Conclusion: Deprived socioeconomic groups have a higher rate of symptomatic carotid disease, but this is not matched by an increased rate of CEA. Socioeconomic inequalities in the prevalence of symptomatic carotid artery disease and treatment in the form of primary and secondary prevention exist

Poster 64

Cytokine gene polymorphisms affect the incidence of organ failure after elective abdominal aortic aneurysm repair

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Aims: Cytokines are important mediators of postoperative organ dysfunction and the sepsis syndrome, both of which frequently complicate abdominal aortic aneurysm (AAA) repair, in addition, polymorphic sites in cytokine genes affect cytokine transcription and may therefore affect outcome in sepsis. The aim of this study was to determine whether cytokine genotype affects outcome after elective AAA repair.

Methods: A prospective study of 84 patients having elective AAA repair was carried out. For each patient, their genotype at the following loci was determined by induced heteroduplex genotyping: IL-1β +3953, IL-6 −174; IL-10 −1082 and −592; and TNF-α −308. The incidence of organ failure, length of critical care stay and length of hospital stay were used as outcome measures.

Results: Total 48.44 per cent of patients with an IL-6 −174 'G' allele (64 patients, 76 per cent) developed organ failure compared to 17.64 per cent of those with no 'G' allele ($P = 0.03$). There was no significant difference in the incidence of organ failure between those patients with high transcription alleles of the IL-1β, IL-10 and TNF-α genes. None of the genes studied had any significant effect on the length of time spent in critical care or total hospital stay.

Conclusion: The incidence of organ failure after AAA repair may be influenced by genetic differences between patients, but these differences do not influence overall outcome.

Poster 65

Minimally invasive incision for abdominal aortic aneurysm repair. Does it have any advantages?

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Aim: To evaluate the feasibility of a minimally invasive incision for the treatment of patients with abdominal aortic aneurysms (AAA).

Methods: Comparative study of 18 patients (nine minimally invasive: MIn; nine conventional: Co) undergoing elective AAA repair. Patients in the MIn group had a 10–12 cm midline incision, whereas those in the Co group had a long midline incision. Both groups were comparable in terms of preoperative morbidity, age and gender. Outcome measures analysed were the C-reactive protein on the first postoperative day, length of operation, blood loss, duration of postoperative epidural, return of GI function and length of hospital stay.

Results: There was one bifurcated graft in the MIn group compared to two in the Co group. For the MIn group, the mean size of aneurysm was 6.2 cm compared to 6.4 cm for the Co group; $P = 0.7$. Mean length of incision was 11.8 cm (MIn) against 28.4 cm (Co); $P = 0.0008$. Mean CRP was 65.5 (MIn) *versus* 90.6 (Co); $P = 0.01$. The length of operation was 127 min (MIn) compared to 150 min (Co); $P = 0.05$. The mean blood loss was 1261 mL (MIn) against 1954 mL (Co); $P = 0.1$. The mean duration of postoperative epidural was 2.3 days (MIn) *versus* 3.7 days (Co); $P = 0.02$. The mean GI function was 2.3 days (MIn) compared to 4.3 days (Co); $P = 0.1$. Hospital stay was 9.7 days (MIn) *versus* 14.7 days (Co); $P = 0.2$.

Conclusion: Elective repair of AAA through a small incision is feasible and seems to have a beneficial effect compared to the traditional repair.

Poster 66

Risk stratification for abdominal aortic aneurysm screening using a novel type III collagen marker

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Aims: The potential benefit of abdominal aortic aneurysm screening is well documented; however, the significant expense and burden on regional radiological services of ultrasonographic screening of asymtomatic patients has been prohibitive to its development. Degeneration of the collagen matrix in the elastic media of major vessels has been implicated in the pathogenesis of aneurysm formation for some time. This study examines whether a novel assay for a degradation product (cross-linked peptide) of type III collagen could permit further risk stratification for the detection of the condition in the community, tipping the balance in favour of a substantial screening programme.

Methods: A total of 32 patients were recruited to the study with a mean age of 69 years. An enzyme-linked immunosorbant assay (ELISA) for a collagen III telopeptide fragment was performed on venous blood samples taken from 12 patients known to have an abdominal aortic aneurysm (greater than 4.0 cm in diameter), 11 control patients without the condition and 9 patients with critical ischaemia of the lower limbs.

Results: The values of the type III collagen degradation product assay in the abdominal aortic aneurysm group were significantly higher ($P = 0.05$; 95 per cent CI: 0.05–4.62) when compared to the control group. There was not a significant difference in the results between the critical ischaemia and control groups nor between the critical ischaemia and abdominal aortic aneurysm groups.

Conclusion: This study demonstrates that this type III collagen degradation marker may have a role in risk stratification for abdominal aortic aneurysm screening.

Poster 67

Patients with critical ischaemia of the lower limb are at risk of developing kidney dysfunction

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Aims: To establish a relationship between critical ischaemia in patients with peripheral arterial occlusive disease and renal tubular dysfunction.

Methods: At rest, urine samples were collected from three groups: group A: 22 patients with stable intermittent claudication; group B: 16 patients with critical ischaemia; group C: 12 normal individuals. All groups were age and sex matched. Urinary *N*-acetyl-β-D-glucosaminidase (NAG) enzyme was measured using a spectrophotometer. Microalbuminuria was also measured. Patients with diabetes or abnormal kidney function were excluded. Results were analysed using Mann–Whitney test and considered significant when $P < 0.05$.

Results: There was a significant difference in the NAG enzyme activity between group B and group A (median: 1.62; IQR: 1.01–4.02 *versus* median: 0.55; IQR: 0.40–0.95; $P = 0.001$). There was a significant difference between group B and group C (median: 1.62; IQR: 1.01–4.02 *versus* median: 0.24; IQR: 0.04–0.77; $P = 0.01$). There was no significant difference between group A and group C (median: 0.55; IQR: 0.40–0.95 *versus* median: 0.24; IQR: 0.04–0.77; $P = 0.13$). Microalbuminuria was not significantly different between the three groups ((median: 7.68; IQR: 1.43–15.50 *versus* median: 2.64; IQR: 1.16–4.82; $P = 0.27$) (median: 7.68; IQR: 1.43–15.50 *versus* median: 1.52; IQR: 0.83–2.20; $P = 0.22$) (median: 2.64; IQR: 1.16–4.82 *versus* median: 1.52; IQR: 0.83–2.20; $P = 0.11$)).

Conclusion: Elevated levels of NAG enzyme in patients with critical ischaemia of the lower limb suggest that they are at greater risk of developing renal tubular dysfunction. This could be caused by the release of more free radicals into the systemic circulation.

Poster 68

Early results of an integrated outpatient deep venous thrombosis (DVT) management service

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Aims: To establish an integrated outpatient DVT management service based in the vascular studies unit in district general hospital and to demonstrate the saving in bed days achieved by such a service.

Methods: Vascular studies unit has been responsible for the diagnosis of DVT in the last few years. Retrospective analysis of workload showed a potential bed saving of 927 bed days in 1 year if patients could be managed without admission to hospital. On the basis of this data, a business case was prepared and accepted by the trust. A new duplex scanner was purchased and one whole time equivalent staff nurse was appointed to run the service. Patients with suspected DVT are referred to the unit by A&E, general practitioners and outpatient clinics, and the duplex scans were performed in the same session. Patients presenting outside office hours are treated with subcutaneous injection of tinzaparin and scanned in the next available session. Patients with DVT are managed according to a strict protocol.

Results: A total of 211 patients with suspected DVT were scanned in the first 3 months. Thirty-four per cent of the patients were already inpatients and were excluded. Of the remaining 139 patients, 44 per cent were referred from GPs, 38 per cent from A&E and 18 per cent from clinics. Eighteen per cent had positive scans and the rest were negative. In patients with DVT, treatment either with tinzaparin or compression therapy initiated in the unit and followed up according to the protocol.

Conclusion: This unique service provides rapid diagnosis and treatment of patients with suspected DVT. In the first 3 months, 331 bed days have been saved.

Poster 69

Residual stenosis after carotid endarterectomy: a comparison of three techniques

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Aims: Direct carotid closure provides inferior outcome compared to patching following carotid endarterectomy, this may be related to the degree of post-operative internal carotid artery stenosis. We evaluated whether eversion endarterectomy gave as good a reduction in stenosis as patching or direct closure following standard endarterectomy.

Method: We reviewed consecutive carotid endarterectomies in a single surgeon practice between November 1995 and December 2001. Three techniques were used: longitudinal arteriotomy with direct closure (D), or patch closure (P) and oblique artery disconnection with eversion endarterectomy (E). D was the main technique for the first 2 years of the study period, being superseded by P and thereafter E. Internal carotid artery stenoses were measured by duplex before and after surgery.

Results: Eighty-eight operations were performed for which 67 postoperative scan results were obtainable. The minimum preoperative and maximum post-operative recorded stenoses were used for analysis. There was one ipsilateral fatal stroke and one nonfatal contralateral stroke in this series.

		Operation type		
		D	E	P
Preoperative	*N*	35	17	35
	Median	75	85	85
	Interquartile range	75–90	75–90	75–90
Postoperative	*N*	27	13	27
	Median	25	20	25
	Interquartile range	25–50	10–25	0–50

There was a significant variation in residual stenosis across the three groups (Kruskal–Wallis test, $P < 0.05$). Pairwise comparison of the three groups showed a significant difference between D and E (Wilcoxon rank sum test, $P < 0.05$ after Bonferroni correction) but not between D and P or between E and P ($P = 0.42$ and $P = 0.47$, respectively).

Conclusion: Eversion endarterectomy provides the least residual stenosis following carotid endarterectomy. Where applicable, it may be the technique of choice.

Poster 70

A call for direct access duplex scan in the diagnosis and management of deep vein thrombosis

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Aims: To evaluate direct access duplex ultrasound scanning in the diagnosis of deep vein thrombosis.

Methods: We offer in our unit, a direct access duplex ultrasound scanning service for the diagnosis of DVT. We reviewed the referral pattern to our service in the last 1 year and the impact on management of DVT.

Results: We performed a total of 965 venous duplex scans for suspected DVT in 1-year period and this included 421 inpatients. The majority of referrals were from accident and emergency (45 per cent), adult medicine (20 per cent) and medical assessment unit (10 per cent). Only 25.6 per cent of patients had femoral and popliteal vein thrombosis and a further 15.8 per cent had calf vein thrombosis. In 56 per cent of patients, there was no abnormality detected. It is calculated that by preventing unnecessary admission of patients with negative scan, there will be a potential reduction in bed occupancy of 927 beds per year and therefore significant cost savings per year.

Conclusions: Outpatient treatment of DVT is now well established. Seventy-five per cent of patients with suspected DVT present through A&E, adult medicine and medical assessment unit. Eighty per cent of these could be treated as outpatient and 20 per cent probably require hospitalization for other associated problems. Direct access duplex service will enable the physician to reach the diagnosis and commence treatment accordingly. It will also prevent unnecessary admission of patients with negative scan. In this scenario, there will be a potential reduction in bed occupancy per year and this could have major impact on efficiency in both medical and surgical directorates.

Poster 71

A randomized, blinded, prospective clinical trial of doxycycline in carotid plaque stabilization

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Aims: Elevated levels of matrix metalloproteinases (MMPs) have been implicated in plaque rupture, particularly MMP-1 and MMP-9. It has been suggested that inhibition of MMPs may stabilize vulnerable atherosclerotic plaques and improve clinical outcome. The aim of the study was to investigate the ability of doxycycline, a nonspecific MMP inhibitor, to reduce MMP concentrations in carotid atheroma.

Methods and Results: Human macrophages were cultured with increasing concentrations of doxycycline, and MMP levels were quantified. A prospective, double-blinded randomized trial was then conducted. One hundred patients requiring carotid endarterectomy were randomized to receive 200 mg doxycycline per day or placebo for 2–8 weeks prior to surgery. Plaques were retrieved and concentrations of MMPs and doxycycline were determined by ELISA and HPLC, respectively. Stroke rate and carotid artery occlusion were used to assess clinical outcome.

Results: Doxycycline inhibited MMP-9 and MMP-1 expression in macrophages in a dose-dependent manner (IC 25 for MMP-1 $= 2.4\ \mu g\ mL^{-1}$, IC 25 for MMP-9 $= 43\ \mu g\ mL^{-1}$). A mean doxycycline concentration of $6.25\ \mu g\ g^{-1}$ of tissue ($SD = 0.29\ \mu g\ mL^{-1}$) was achieved. There was a significant reduction of MMP-1 levels in the active group compared with placebo (mean: $11.52\ ng\ mL^{-1}$, SD: 10.28, 22.26, $45.64\ ng\ mL^{-1}$, respectively; $P = 0.035$). RT-PCR showed reduced MMP-1 mRNA expression in active *versus* placebo (mean optical density: 0.23 ± 0.06 (SD) *versus* 0.42 ± 0.02 (SD), respectively, $P = 0.02$). There were four events in the placebo group compared to one event in the active group ($P =$ ns).

Conclusions: Doxycycline penetrated atherosclerotic plaques with acceptable tissue levels. This resulted in a reduction in MMP-1 concentration due to decreased expression.

Poster 72

The unmet need for carotid endarterectomy: where are we failing?

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Aims: Not all patients with appropriate symptomatic carotid stenosis are referred to vascular surgeons, and in 2001 we performed half the expected number of carotid endarterectomies for our population. We audited the primary care management and pattern of referral for patients with symptomatic cerebrovascular disease in our region.

Methods: A questionnaire was sent to 240 local GPs and 20 casualty officers regarding their management of patients presenting with amaurosis fugax, a transient ischaemic attack (TIA) or collapse. Outcome measures included cardiovascular risk factor modification, antiplatelet prescription and referral for specialist opinion.

Results: Seventy-three per cent of GPs and 75 per cent of casualty officers responded. Cardiovascular risk factors were addressed by all doctors: 100 per cent measured blood pressure, 91 per cent measured fasting lipids, 88 per cent measured serum glucose, and 98 per cent advised smokers to stop. Ninety-five per cent prescribed an antiplatelet agent, the majority using aspirin. A total of 160 GPs (91 per cent) and nine casualty officers (60 per cent) requested a specialist opinion which are detailed in the Table.

	Number of patients (%) with symptoms		
Speciality	Amaurosis fugax	TIA	Collapse ?cause
Vascular surgeon	56 (33%)	26 (15%)	1 (0%)
Neurologist	56 (33%)	76 (45%)	30 (18%)
General physician/geriatrician	49 (29%)	63 (37%)	120 (71%)
Cardiologist	1 (0%)	2 (1%)	12 (7%)
Other	8 (5%)	2 (1%)	6 (4%)

Conclusions: Patients with symptomatic cerebrovascular disease are being referred to specialists, but to physicians, not surgeons. Poor communication between hospital specialities may underlie the unmet need for carotid endarterectomy in our region, suggesting that a more integrated multidisciplinary approach to cerebrovascular disease is required.

Poster 73

A comparison of physiology scores and morphology in a group of patients evaluated for endovascular repair of infrarenal aneurysms

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Aims: To maximally reduce the mortality of abdominal aortic aneurysm (AAA) repair, endovascular repair (EVR) would have to be technically possible in high-risk patients unsuitable for open repair (OR). We set out to test this hypothesis.

Methods: Anatomical suitability and POSSUM scores were compared in 196 patients presenting for AAA repair. Patients were evaluated by CT scan and angiography. The threshold for repair was 55 mm.

Results: Mean age was 74.5 years (54.1–89.2), and 163 (83 per cent) were male. A basic bifurcated graft could have been used to treat 33 (17 per cent) patients (group A), 88 patients could have been treated by EVR by a more complex graft (group B) and 108 were unsuitable for EVR. In the whole group, mean AAA diameter was 60.5 mm. More unsuitable aneurysms were larger than this (62/108) in either group A (11/33; $P < 0.02$) or group B (34/54; $P < 0.01$). The median of the physiology component of POSSUM scores was 19 (interquartile range: 17–21) in the whole group, 18 (17–19) in group A, 18 (17–21) in group B and 19 (18–21) in those unsuitable for EVR. Of 133 AAA ^{3}55 mm, physiology scores were greater than the median in 56/133 of the whole group, 4/19 in group A, 16/51 in group B and 40/42 in those unsuitable for stenting ($P < 0.05$ for group A *versus* unsuitable and $P < 0.05$ for group B *versus* unsuitable).

Conclusions: Larger aneurysms are less likely to be anatomically suitable for EVR. Ironically, those in whom EVR would appear to be most appropriate, the physiologically less fit, are more likely to be anatomically unsuitable for EVR.

Poster 74

Ischaemic reperfusion injury following percutaneous transluminal angioplasty in patients with peripheral arterial occlusive disease

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Aims: The exact mechanism of limb swelling after successful revascularization by percutaneous transluminal angioplasty (PTA) in patients with peripheral arterial insufficiency remains uncertain. The aim of this study was to investigate the probable cause(s) of limb swelling in these patients.

Methods: Thirty patients with peripheral arterial occlusive disease (PAOD) undergoing PTA were selected. Each patient was subjected to limb-volume measurement using perometer, ABPI and serum interleukin-6 (IL-6) measurements using a sensitive enzyme-linked immunosorbent assay preoperatively and postoperatively at 6–8 days. Revascularization was considered successful when there was symptomatic improvement with accompanying rise in ABPI and increase in claudication distance.

Results: The mean change of postoperative volumes was 11.3 per cent for successful angioplasty, whereas in patients with failed angioplasty ($n = 10$) volume change was 4.2 per cent. The corresponding increase in the levels of serum IL-6 was 41.9 pg mL^{-1} for successful and 3.3 pg mL^{-1} for the failed angioplasty patients. Our previous study has shown that maximum limb swelling occurs at 6–8 days postoperatively. The present study confirms significant limb swelling ($P > 0.001$) after successful revascularization with PTA. There was also a significant ($P < 0.001$) increase in the level of serum IL-6 following successful revascularization, whereas serum IL-6 changes in failed angioplasty were unremarkable.

Conclusions: This study shows that there is limb swelling following successful revascularization by PTA and serum IL-6 measurements implicate reperfusion injury as the most probable cause of limb swelling. These findings may help to understand the complex mechanism of ischaemic reperfusion injury phenomenon in patients with PAOD undergoing revascularization.

Poster 75

Abdominal aortic aneurysm repair in the octagenarian. Three-year cumulative survival from a single centre

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Aims: Abdominal aortic aneurysm (AAA) repair in the patients over 80 years of age can be associated with acceptable in-hospital mortality, but the longer term survival of these patients is often unclear. The aim of this study was to determine the 3-year cumulative survival for all octagenarian patients undergoing AAA repair (elective and emergency) at a single centre during a 46-month period.

Methods: All patients aged 80 years or above who underwent AAA repair between January 1996 and November 1999 were identified from a prospectively maintained vascular database. In-hospital mortality was calculated and a telephone survey used to assess longer term outcome. Thirty-six month cumulative survival was determined using Kaplan–Meier survival curves.

Results: During this period, 47 octagenarian patients (median age: 82 years, M:F = 36:11) underwent surgical repair of AAA. Twenty-six procedures were performed electively and 21 as an emergency (following rupture). The in-hospital mortality was 11.5 per cent (3/26) for the elective group and 38.1 per cent for the emergency group (8/21). For patients discharged from hospital, the 36-month cumulative survival was 78 per cent.

Conclusions: These data show that AAA repair in octagenarian patients is associated with an acceptable in-hospital mortality and that the majority of surviving patients will still be alive after 3 years. We would therefore conclude that AAA surgery should not be withheld on the basis of age alone.

Poster 76

Multiple cross-sectional echomorphology: a new method for identifying high-risk carotid plaques

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Aims: Although single longitudinal view (SLV) ultrasound echomorphology is said to identify high-risk carotid plaques for which selective endarterectomy might improve surgical risk · benefit, it only examines a limited, operator-dependent (potential sampling errors) plaque area, and fails to convincingly separate echolucency from per cent carotid stenosis as an independent risk factor. Thus, a new method of multiple cross-sectional imaging (MCSI) has been developed and its ability to distinguish recently symptomatic from asymptomatic plaques assessed.

Methods: Seventy-four bifurcation carotid stenoses (70–99 per cent, 59 patients 33 asymptomatic; 41 symptoms within 3 months) were imaged (Acuson 128-10XP) to obtain the 'best' SLV and 6–8 MCSI images along their length. Digitized sonograms were computerized and normalized to the grey scale median (GSM) of blood and vessel adventitia. Plaque GSM (echogenicity) was determined for SLV and for each MCSI image (analysis MATLAB 5.3). SLV GSM, minimum MCSI GSM (image with lowest GSM) and cross-sectional axial heterogeneity (highest–lowest MCSI GSM) were compared in the groups.

Results: The percentage of stenosis was similar in both groups as was SLV GSM (median, i.q.r.) symptomatic 34 (24.8–43); asymptomatic 43 (32.6–53.2), $P = 0.1$. The minimum MCSI GSM was lower in symptomatic plaques (7.0 (4.2–9.8) *versus* 18.3 (12.2–24.5); $P = 0.002$) which also showed greater cross-sectional heterogeneity (34.5 (27.2–41.9) *versus* 16 (11–20.8), $P = 0.0001$)

Conclusions: MCSI is more sensitive than SLV sonography in assessing plaque echolucency and also appraises plaque heterogeneity (not possible with SLV), thus distinguishing high-risk symptomatic from low-risk asymptomatic plaques. This novel technique should be more effective than SLV measurements in improving the risk · benefit of CEA.

Poster 77

A new technique for carotid endarterectomy allowing duplex quality control and enhanced plaque research

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Aims: Although selection criteria for carotid endarterectomy (CEA) were established angiographically, most centres now rely upon operator-dependent duplex ultrasound for advising surgery. In view of this, we have developed a modified CEA technique which provides intact specimens allowing duplex quality control and, unlike conventional endarterectomy (plaque incised), an intact plaque for atherosclerosis research.

Methods: After exposure and clamping of the carotid vessels, a 5-mm common carotid arteriotomy allows identification of the endarterectomy plane. This is developed distally with a dissector positioned superficial to the unopened plaque and deep to the uncut media-adventitia. The dissector is then advanced to the distal endarterectomy limit and the entire, intact, plaque exposed and removed following longitudinal division of the media-adventitia. CEA is performed in 2–3 min allowing shunt insertion (if required) after plaque removal. The specificity of duplex velocity criteria (DVC) for >70 per cent stenoses (ICA PSV > 180 cm s^{-1}, ICA EDV > 75 cm s^{-1} and ICA PSV/CCA PSV > 3) has been assessed against computer-digitized images of 5 µ stained (H&E, EVG) sections cut at 2 mm intervals throughout the length of 21 consecutive CEA specimens which allowed calculation of per cent diameter stenosis (ECST method).

Results: Histology confirmed preservation of normal plaque architecture in the specimens. DVC correctly identified a >70 per cent stenosis in 19/21 plaques (specificity: 90.4 per cent) compared to specimen measurements and 2/21 stenoses measured 58 and 64 per cent. The correlation between specimen and duplex assessment for each plaque was $r = 0.78$ and $P < 0.0001$.

Conclusions: This modified CEA technique provides an ideal method for quality control of vascular laboratory carotid imaging and for obtaining atherosclerosis research material.

Poster 78

Increased inducible nitric oxide synthase and matrix metalloproteinase MMP-9 in the symptomatic carotid artery plaque

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Aims: Differences in the natural history of symptomatic and asymptomatic carotid stenosis may be reflected in changes in plaque biology.

Methods: Following ethical approval, carotid artery plaques were obtained from symptomatic ($n = 34$) or asymptomatic ($n = 10$) patients with internal carotid artery stenosis of greater than 70 per cent. These were stained for the presence of the inducible (iNOS) and gelatinase matrix metalloproteinase (MMP-9). Areas of staining for the whole plaque were related to the presence or absence of symptoms, and the time between the acute event and endarterectomy. Analysis was performed by two independent observers, blinded to the patients' symptoms, using the analySIS computer programme.

Results: The study comprised 44 patients of whom 32 were male (mean age: 70.1, range: 50–83). *P*-values are for Mann–Whitney nonparametric tests, all units are in µm^2 expressed as a median (interquartile range)

	Asymptomatic $n = 10$	Symptomatic $n = 34$	*P*-value
Total iNOS	7.28 (0–9.77)	18.76 (8.35–22.28)	0.006
Total MMP-9	8.13 (5.18–15.3)	19.33 (12.62–24.78)	0.006
Cap iNOS	0 (0–7.81)	6.91 (0–10.05)	0.04
Core iNOS	1.89 (0–7.62)	5.935 (0–9.09)	NS 0.3
Shoulder iNOS	0 (0–0)	1.87 (0–8.87)	0.007
Cap MMP-9	0 (0–7.76)	7.76 (0–12.12)	0.03
Core MMP-9	5.83 (0–7.62)	6.29 (4.11–9.99)	NS 0.3
Shoulder MMP-9	0 (0–0)	5.53 (0–8.37)	0.01

Conclusions: Staining for iNOS and MMP-9 is significantly increased in the cap and shoulder of carotid plaques from symptomatic patients. This may represent a model for the mechanism of plaque rupture.

Poster 79

A randomized study of once weekly supervised exercise motivation class *versus* exercise advice alone for the treatment of intermittent claudication (3-month results)

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Aims: Exercise advice is the main treatment for symptom relief in patients with mild to moderate intermittent claudication (IC). Would a weekly exercise/motivation class offer adjuvant benefit to claudicants?

Methods: Fifty-six patients attending a regional vascular centre for whom IC was the main factor affecting mobility were randomly allocated to either exercise advice alone ($n = 28$) or exercise advice with a once a week 45-min supervised exercise/motivation class ($n = 28$). Mean age was 68 years (±SD of 9 years), 73 per cent being male. Baseline and 3-month assessment included absolute claudication time (ACT) on fixed load treadmill (3.5 km h^{-1}; 12 per cent), quality-of-life questionnaires and the 6-min walk test (SMW)

Results: Both the exercise class and the advice-only groups had significant increases in mean ACT, from 142 to 248 s ($P = 0.0002$) and from 135 to 157 s ($P = 0.018$), respectively. An analysis of variance adjusting for age, sex, and baseline ACT showed 75 per cent improvement in the class group to be significantly better than the 16 per cent improvement in the advice-only group ($P = 0.05$). A similar analysis of variants performed for the SMW showed a significant 8 per cent improvement from 450 to 487 min in the class group compared with a 4 per cent increase from 404 to 419 min in the advice-only group ($P = 0.03$).

Conclusions: A weekly supervised exercise and motivation class, for a 3-month period, shows a significant improvement in walking ability compared to advice alone.

Poster 80

Critically ischaemic feet harbour an endothelium mediated but nitric oxide independent pathway that shunts blood away from the nutritional microcirculation

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Aims: Technically successful arterial bypass grafts for critical lower limb ischaemia (CLLI) are often complicated by chronic limb oedema, infection and occlusion. We aimed to investigate whether changes in the microcirculation may account for these.

Methods: Comparison of supine foot cutaneous microvascular reactivity was made between controls, group 1, palpable foot pulse, and patients with CLLI, group 2, rest pain and/or tissue loss. Vasodilatation responses to iontophoretically delivered acetylcholine chloride (ACh, endothelium dependent) and sodium nitroprusside (SNP, endothelium independent) were measured in perfusion units (PU), using laser Doppler fluximetry. Results were compared using Student's *t*-test.

Results: Groups 1 ($n = 10$) and 2 ($n = 20$) were similar, with mean age of 73.6 years (range: 57–87; 5 M, 5 F) and 75.9 years (range: 57–90; 11 M, 9 F), respectively. Baseline blood flow was higher in group 2 than in group 1 (mean: 11.86 ± 5.95 PU *versus* 7.6 ± 2.83 PU; $P < 0.05$). The group 2 SNP response was 24 per cent of the group 1 response (9.24 ± 22.08 PU *versus* 37.97 ± 44.19 PU; $P = 0.039$), whereas the magnitude of the group 2 ACh response was less impaired, reduced to 74 per cent of group 1 (24.38 ± 47.64 PU *versus* 33.68 ± 32.64 PU; $P = 0.151$).

Conclusions: These data suggest that in CLLI, ACh-mediated smooth muscle relaxation may be mediated through a nitric oxide (NO)-independent pathway, resulting in the inappropriate shunting of blood flow away from the nutritional circulation. This shunting process may explain some of the poor responses to revascularization and offers the surgeon a target for adjuvant pharmacotherapy.

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